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ANTIGENIC CONSTRUCTS OF MAJOR HISTOCOMPATIBILITY COMPLEX CLASS 1 ANTIGENS WITH SPECIFIC CARRIER MOLECULES, THE PREPARATION AND USE THEREOF

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(71) Applicant(s)
BEHRINGWERKE AKTIENGESELLSCHAFT

(72) Inventor(s)
GERHARD SEEMANN; KLAUS BOSSLET; HANS HARALD SEDLACEK

(74) Attorney or Agent
WATERMARK PATENT & TRADEMARK ATTORNEYS , Locked Bag 5, HAWTHORN VIC 3122

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(57) It has been found that target-cell-specific carriers, for example preferably monoclonal antibodies (mAb), but also polyclonal antibodies or molecules which bind to receptors on cells, can be coupled to the N- or C-terminal end of an allogenic MHC class I molecule without this altering the allogenic determinants in a disadvantageous manner. The MHC class I molecule is brought, with the aid of this target-cell-specific carrier, specifically to the target cells, which leads to activation of allospecific T cells and thus to destruction of the target cells by allospecific cytotoxic T cells. One explanation for the success of the coupling of a target-cell-specific carrier to the N- or C-terminal end of a MHC class I molecule while retaining the allogenic determinants is that the N-terminal end of the MHC class I molecule is located on the side of the $alpha_1$ and $alpha_2$ domains which points towards the cell, whereas the allogenic determinants are located on the side of the $alpha_1$ and $alpha_2$ domains which faces away from the cell (Fig. 1, Fig. 34).

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Claim

1. Antigenic constructs adapted to induce the specific activation of allospecific T cells and in which major histocompatibility complex (MHC) class I antigens are linked at the C- or N- terminal end to specific carrier molecules.

7. Antigenic constructs as claimed in claim 6, in which the monoclonal antibodies are shortened in the constant part of the heavy chain.

8. Antigenic constructs as claimed in claim 1, 2, 3, 4, 5, 6 or 7, in which the MHC class I antigen is HLA B27w or HLA B27k.

12. A process for the preparation of antigenic constructs as claimed in claim 1, 2, 3, 4, 5, 6, 7, 8 or 9, which comprises the required parts of genes being fused in the form of their DNA, being provided with suitable regulation sequences and being expressed in suitable expression systems.

14. A pharmaceutical which contains antigenic constructs as claimed in any one of claims 1 to 11.

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Form 10

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Name of Applicant : BEHRINGWERKE AKTIENGESELLSCHAFT

Address of Applicant : D-3550 Marburg, Federal Republic of Germany

Actual Inventor: GERHARD SEEMANN, KLAUS BOSSLET, HANS HARALD SEDLACEK

Address for Service : [redacted]Watermark Patent & Trademark Attorneys
50 QUEEN STREET, MELBOURNE, AUSTRALIA, 3000.

Complete Specification for the invention entitled:

ANTIGENIC CONSTRUCTS OF MAJOR HISTOCOMPATIBILITY COMPLEX CLASS I ANTIGENS WITH SPECIFIC CARRIER MOLECULES, THE PREPARATION AND USE THEREOF

The following statement is a full description of this invention, including the best method of performing it known to :- us

BEHRINGWERKE AKTIENGESELLSCHAFT

88/B 019 - Ma 706
Dr. Lp/rd

Antigenic constructs of major histocompatibility complex class I antigens with specific carrier molecules, the preparation and use thereof

The invention relates to antigenic constructs resulting from the linkage of major histocompatibility complex (MHC) class I antigens with specific carrier molecules.

Tissue-rejection reactions are the strongest-known immune responses mediated by T cells. In individuals of the same species they are caused by allogenic differences in class I and class II MHC antigens. In organ transplants, for example, any allogenic determinants of the MHC antigens present in the donor tissue are recognized as foreign by allospecific T cells of the recipient, a T cell immune response is induced, and the rejection reaction takes place unless an immunosuppressive therapy has been initiated or such a therapy proves insufficient.

It is furthermore known that MHC class I antigens are glycoproteins which are expressed on the surface of all nucleated cells. They are composed of a heavy chain, which is encoded by MHC class I genes, and of a light chain, the β_2-microglobulin which is non-covalently associated with the heavy chain. The extracellular part of the heavy chain is folded in three domains, the first two of these domains (alpha$_1$ and alpha$_2$) exhibiting a pronounced polymorphism when the amino acid sequences of hitherto known class I MHC antigens from various individuals are compared. They assist with antigen presentation and carry the allogenic determinants. The third extracellular domain has a more conserved sequence. The association with β_2-microglobulin is essential for correct folding of the heavy chain and for the transport of the molecule to the cell surface.

Isolation and characterization of mutated MHC class I antigens in mice showed that merely a few differences in amino acids on the alpha$_1$ and alpha$_2$ domains between donor and recipient suffice to induce a rejection reaction (Mathenson et al., Ann. Rev. Immunol., 1986, 4, 471-502). It has also been shown in humans that slight differences between donor and recipient lead to rejection of a transplant (Dausset, J., Rapaport, F.T., Legrand, L., Colombani, J., Marcelli-Barge, A.: Skin allograft survival in 238 human subjects: Role of specific relationships at the four gene sites of the first and the second HL-A loci., Histocompatibility Testing (1970) pages 381-397, Terasaki P.I. (Ed.)). The task which presented itself from that said above was to utilize the specific inducibility and strength of the cellular immune response in the tissue-rejection reaction to damage or destroy selected target cells.

It has been found that target-cell-specific carriers, for example preferably monoclonal antibodies (mAb), but also polyclonal antibodies or molecules which bind to receptors on cells, can be coupled to the N- or C-terminal end of an allogenic MHC class I molecule without this altering the allogenic determinants in a disadvantageous manner. The MHC class I molecule is brought, with the aid of this target-cell-specific carrier, specifically to the target cells, which leads to activation of allospecific T cells and thus to destruction of the target cells by allospecific cytotoxic T cells. One explanation for the success of the coupling of a target-cell-specific carrier to the N- or C-terminal end of a MHC class I molecule while retaining the allogenic determinants is that the N-terminal end of the MHC class I molecule is located on the side of the alpha$_1$ and alpha$_2$ domains which points towards the cell, whereas the allogenic determinants are located on the side of the alpha$_1$ and alpha$_2$ domains which faces away from the cell (Fig. 1, Fig. 34).

Because of the great polymorphism of the MHC class I

antigens in the human population, it is possible to induce a rejection reaction in almost 100 % of the population with the aid of only two different MHC class I molecules selected, for example HLA B27w and HLA B27k. HLA B27w and HLA B27k are two subtypes of the serologically defined HLA B27 specificity which are defined by cytotoxic T lymphocytes. In the caucasoid population about 7 % of individuals express HLA B27. and about 1 % express HLA B27k. The use of, for example, both HLA B27 subtypes for the allogenization in accordance with this invention makes it possible to treat almost 100 % of the caucasoid population. However, it is possible according to the invention to couple any desired MHC class I antigen to the relevant specific carriers if the above-mentioned antigens do not lead in the relevant recipient to activation of allospecific T cells and subsequent damage to or destruction of the target cells. Target cells may be regarded as cells which are undesired and/or pathogenic in the body, such as, for example, tumor cells. The antigenic constructs according to the invention are accordingly suitable for tumor therapy. However, it is also possible with the MHC class I antigenic constructs according to the invention to treat other diseases which are caused by cells or the products thereof and are favorably affected by elimination of these cells. The mode of action of the hybrid molecules described in example groups I and II derives from the fact that they are able, because of the specifity of the antibody portion, to bind to an antigen on the cell. The HLA B27 portion of the fused molecule results in masking of the surface of the target cell with an allogenic MHC class I molecule. These allogenic class I molecules can then be recognized by syngeneic, allospecific, cytotoxic T cells, which leads to destruction of the target cells by the allospecific cytotoxic T cells. Accordingly, the invention relates to

a) MHC class I antigens which are linked N- or C-terminally to specific carriers, the linkage

preferably being brought about covalently but also possibly being non-covalent, for example by a biotin-avidin bridge, and the specific carriers binding selectively to target cells and denoting preferably monoclonal, but also polyclonal, antibodies, but being very generally receptor-binding molecules which bind to the particular cell receptors,

b) a process for the preparation of the MHC class I antigenic constructs, and

c) the use of the MHC class I antigenic constructs mentioned in a) and b) for damaging or eliminating target cells.

The invention is furthermore described in the examples which follow and in the patent claims, but it is not to be regarded as restricted thereto.

Examples 1 - 17 detailed hereinafter describe a construct according to the invention composed of the nitrophenol (NP)-specific mouse mAb B/1-8 V_H gene (1), of a human IgG C $F(ab')_2$ gene (2) and of an HLA B27w gene (3). (1) and (2) are to be regarded in this context as examples of the specific carrier portion - in this case an mAb against NP - whereas (3) represents an HLA class I antigen.

The abovementioned construct is, after appropriate transformation, expressed and secreted by those myeloma cells which contain a human β_2-microglobulin and a light chain of an immunoglobulin and whose V gene forms with V_H B/1-8 a NP binding-site, such as, for example, the mice myeloma cell J 558 L (Oi, V.T., Morrison, S.L., Herzenberg, L.A., Berg, P.: Immunoglobulin gene expression in transformed lymphoid cells. Proc. Natl. Acad. Sci. USA 80, 825, 1983). It is possible, by exchanging the V_H gene of the heavy chain and using an appropriate light chain, to provide the mAb/HLA B27w fusion product

with any desired specificity for which a specific or selective mAb exists.

Examples

I Examples 1 to 13 show the construction of an HLA B27/mAb fusion gene with the HLA B27 portion at the 3' end of the monoclonal antibody

A) Preparation of the mAb C gene portion (IgG_3 C gene)

Example 1

A human IgG_3 C gene was isolated from a human gene bank in EMBL3 phages (Frischauf, A.-M., Lehrach, H., Proustka, A., Murray, N.: Lambda replacement vectors carrying polylinker sequences. J. Mol. Biol. 170, 827-842 (1983) and Seemann, G.H.A., Rein, R.S., Brown, C.S., Ploegh, H.L.: Gene conversion-like mechanisms may generate polymorphism in human class I genes. The EMBO Journal 5, 547-552 (1986)) and subcloned as a HindIII/Sph I fragment 3.1 kb in size into the plasmid vector pUC 19 (clone 54.1.24) (Fig. 2).

All the techniques used in these and in the following examples were taken, unless otherwise indicated, from Lehrach, H. and Frischauf, A.-M. Laboratory Manual EMBL (1982), Heidelberg; Maniatis, T., Fritsch, E.F., Sambrook, J.: Molecular Cloning: A laboratory manual (1982), Cold Spring Harbor Laboratory.

Example 2

The 54.1.24 clone was subjected to complete HindIII and partial PstI restriction digestion. This results, inter alia, in restriction fragments which contain the C_m exon and one, two or three hinge exons. These fragments were cut out of an agarose gel and cloned into a pUC 19 vector cut with HindIII and PstI (Fig. 3).

The plasmid clone with the $C_{\gamma 1}$ and three hinge exons ($F(ab')_2$ 3H) was then cleaved with BamHI and Asp 718, the cleavage sites were filled in and religated with T_4 ligase (Fig. 4). This deletes the pUC 19 polylinker between the Xba I and the SstI cleavage site.

I B) Preparation of the HLA B27 gene

Example 3

An HLA B27w gene was isolated from a genomic gene bank cloned in EMBL3 bacteriophages (Frischauf, A.-M., loc. cit., and Seemann, G.H.A., loc. cit.) and characterized by restriction mapping and nucleotide sequence analysis (Maxam, A., Gilbert, W.: Sequencing end-labeled DNA with base specific chemical cleavage. Meth. Enzymol. 65, 499-560 (1980) and Sanger, F., Nicklen, S., Coulson, A.R.: DNA sequencing with chain terminating inhibitors. Proc. Natl. Acad. Sci. USA 74, 5463-5471i (1977)) (Fig. 5).

The HLA B27w gene was then digested with the restriction enzymes SstI and BglII and subcloned into the SstI and BamHI cleavage sites of pUC 19. Plasmid clones with the subfragments A, B and C (Fig. 5) were isolated.

Example 4

The plasmid with subfragment A was cleaved completely with SstI and partially with SmaI and, after fractionation on an agarose gel, the fragment A' (Fig. 6) was cloned in a pUC 19 plasmid cleaved with HincII and SstI.

Example 5

The plasmid with the subfragment B was digested with XbaI, and the resulting XbaI insert (B') was cloned in a XbaI-cleaved pUC 19 plasmid (Fig. 7).

Example 6

The plasmid with the subfragment C was cleaved completely with HindIII and partially with SstI, and the fragment which, in the HLA B27w gene, is attached to fragment A (C') was, after fractionation on an agarose gel, isolated and cloned into the Bluescript KS+ phasmid vector (Stratagene, LaJolla, CA, USA) cleaved with HindIII and SstI (Fig. 8).

Example 7

Single-stranded phages were prepared from the KS+ phasmid vector C' by infection with VCS-M13 helper phages (Stratagene, Cat # 200251) and were purified (Stratagene: Bluescript Exo/Mung DNA sequencing system: Instruction Manual). A synthetic oligonucleotide (I = 5'CCTTACCTCATCTCAGG3') was hybridized onto these single strands, and the remainder of the second strand was synthesized using Klenow polymerase. The double-stranded phasmids generated in this way were transformed into XL Blue bacteria and then single-stranded phages were again generated from the resulting plasmid clones by infection with helper phages, and the nucleotide sequence was determined with the aid of an oligonucleotide primer II (5'TGAGGGCTCCTGCTT3') (Sanger, F. et al., loc. cit.). A clone in which the codon TGG (amino acid 274) at the 3' end of the alpha3 exon had been mutated to a stop codon (TGA) was identified (C") (Fig. 9).

Example 8

The plasmid with the fragment A' was cleaved with SstI and ligated with the C" fragment which had been generated by a complete HindIII and partial SstI cleavage of the phasmid clone C" and had been isolated after fractionation on an agarose gel. After ligation at 14°C for 30 minutes, the unligated ends were filled in with T_4 polymerase and subsequently ligated once again. Restriction mapping was

used to identify the plasmid D (Fig. 10) in which the fragment A' is connected to the fragment C' via the SstI cleavage site in the alpha2 exon.

Example 9

The plasmid with the fragment D was cleaved with XbaI and ligated with the fragment B' which had been cut out of the plasmid B' with XbaI and had been purified after fractionation on an agarose gel (Fig. 11). Nucleotide sequence analyses (17) were used to identify a plasmid (E) in which the B' fragment is ligated in the correct 5'-3' orientation to the fragment D.

C) Fusion of the modified HLA B27w gene with the IgG3 C $F(ab')_2$ 3H gene fragment

Example 10

The fragment E was cut out of the plasmid E by cleavage with EcoRI and HindIII, the ends were filled in with T_4 polymerase and purified after fractionation on an agarose gel. This purified fragment E was then ligated with the plasmid which contains the IgG3 $F(ab')_2$ 3H fragment after the latter had been cleaved and the XbaI ends had been filled in with T_4 polymerase (Fig. 12). Restriction mapping was used to identify the clone which contained the plasmid F, in which the modified HLA B27w gene is fused in the correct 5'-3'orientation to the $F(ab')_2$ 3H gene.

Example 11

The fragment F was cut out with HindIII and EcoRI in order to place a polylinker in front of the 5' end of the fragment F. The HindIII and XbaI ends were filled in with T_4 polymerase and cloned into a pUC 19 which had been cleaved with SstI and whose SstI ends had been filled in with T_4 polymerase. Restriction analyses were used to

identify the clone with the plasmid G which has the pUC 19 polylinker 5' from the fragment F (Fig. 13).

Example 12

The plasmid G was cleaved with HindIII and EcoRI, and the insert with the IgG F(ab')$_2$ HLA B27w fusion gene was isolated and cloned into a Bluescript KS+ phasmid vector (Stratagene: Bluescript Exo/Mung DNA sequencing system: Instruction Manual) cleaved with HindIII and EcoRI (Fig. 14).

Example 13

The plasmid H resulting from this cloning was then cleaved with BamHI, and the insert was cloned into the eukaryotic expression vector pEV$_B$ (Simon, T., Rajewsky, K., Nucl. Acids Res. 16, 354, (1988), which contains the IgG H promoter/enhancer sequences and the V$_B$ gene originating from the NP-specific mouse mAb B/1-8 which had been cleaved with BamHI (Fig. 15) (Neuberger, M.N.: EMBO Journal 2, 1375-1378 (1983)). Restriction analysis was used to identify the plasmid I in which the IgG 3 F(ab')$_2$ HLA B27w fusion gene is cloned in the correct 5'-3' orientation behind the V$_B$ gene.

The mAb/HLA B27w fusion gene now possesses intact 5' and 3' ends having all the signals required for expression in eukaryotic cells. The construct is, as stated in the introduction, expressed and secreted in every myeloma cell which contains a human β_2-microglobulin and a light chain of an immunoglobulin and whose V gene forms with V$_B$ B/1-8 a NP binding site, such as, for example, the mouse myeloma cell J 558L (Oi, V.T., Morrison, S.L., Herzenberg, L.A., Berg, P., Proc. Natl. Acad. Sci. USA 80, 825 (1983)).

II Examples 14 to 17 show the construction of an HLA B27/mAb fusion gene with the HLA B27 portion at the

5' end of the monoclonal antibody

A) Preparation of the HLA B27 gene

Example 14

An HLA B27w gene was isolated from a genomic gene bank cloned in EMBL3 bacteriophages (Frischauf et al., loc. cit. and Seemann, G.H.A., loc. cit.) and characterized by restriction mapping and nucleotide sequence analysis (Maxam et al., loc. cit. and Sanger et al., loc. cit.) (Fig. 5).

The HLA B27w gene was then digested with the restriction enzymes SstI and BglII and subcloned into the SstI and BamHI cleavage sites of pUC 19. Plasmid clones with the subfragments A, B and C (Fig. 5) were isolated.

The plasmid with the HLA B27 subclone C was partially cleaved with PstI. The protruding 3' ends of the PstI cleavage sites were removed with T_4 polymerase, with addition of dGTP, and religated with T_4 ligase. Restriction analysis was used to identify the plasmid clone C1 which contains no PstI cleavage site in the intron between the alpha2 and alpha3 exon (Fig. 16).

The plasmid clone C1 was cleaved partially with SstI and completely with HindIII, and the C1' fragment was isolated and cloned into a double-stranded M13 mp18 vector cleaved with SstI and HindIII. The M13 clone C1' with the C1' fragment was identified by determining the nucleic acid sequence of the insert (Fig. 17).

Using the protocol of the Bio-Rad Muta-Gene M13 mutagenesis kit there were isolated from the C1' M13 mp18 phages in the bacterial strain CJ236 single-stranded phages which contained uracils. An oligonucleotide (oligonucleotide III) with the sequence 5' GCGCGCTGGAGCGTCTC 3' was hybridized onto these single-stranded phages and the

second strand was synthesized with addition of T_4 polymerase, dNTP and T_4 ligase.

After infection of the bacterial strain MV 1190 the mutated clone C2 was identified by restriction analysis of the M13 mp18 double-stranded DNAs and confirmed by nucleic acid sequence analysis (Fig. 18). The mutagenesis resulted in destruction of the PstI restriction cleavage site in the alpha2 exon without altering the reading frame or the encoded amino acid sequence.

Single-stranded phages were in turn produced from the M13 clone C2 in the bacterial strain CJ236 and were hybridized with the oligonucleotide IV (oligonucleotide IV = 5'GGGGACGGTGGAATTCGAAGACGGCTC3'). The second strand was then synthesized with T_4 polymerase, T_4 ligase and dNTP. After transformation into MV 1190 bacteria, the M13 mp18 clone C2' was identified by restriction analysis, and the mutation was verified as correct by nucleotide sequence analysis (Fig. 19). This mutagenesis resulted in an EcoRI and an AsuII cleavage site being introduced into the TM exon of the HLA B27 gene, and in amino acid 279 being converted from glutamine into asparagine (Fig. 20).

Example 15

The plasmid clone with the subfragment B was digested with XbaI, and the resulting XbaI insert (B') was cloned in an XbaI-cleaved pUC 19 plasmid (Fig. 7).

The plasmid clone with the fragment A was cleaved completely with HindII and partially with SmaI, and religated. Restriction analysis was used to identify the clone A' in which part of the pUC 19 polylinker is deleted (Fig. 21).

The plasmid clone A' was cleaved partially with SstI and ligated with the C2 fragment generated by an SstI cleavage of the plasmid clone C2 and isolated after

fractionation on an agarose gel. Restriction mapping was used to identify the plasmid D_1 (Fig. 22) in which the fragment A is connected to the fragment C2 via the SstI cleavage site in the alpha2 exon. The 5' end of the HLA B27w gene is thus complete.

Construction of the linker:

Two oligonucleotides were synthesized:

oligonucleotide Va:

5' TCGAATTCCG GCGAGGCAGC TCCCGCAGCT GCACCCGCAG CAGCCGCAGC
AGGCGGGCAG GTCCAACTGC AGGA 3'

oligonucleotide Vb:

5' TCCTGCAGTT GGACCTGCCC GCCTGCTGCG GCTGCTGCGG GTCCAGCTGC
GGGAGCTGCC TCGCCGGAAT TCGA 3'

The two oligonucleotides were hybridized together. This resulted in double-stranded DNA fragments with an EcoRI restriction cleavage site at one end and a PstI restriction cleavage site at the other end. These fragments were cleaved with EcoRI and PstI and cloned into an EcoRI- and PstI-cleaved pUC 19 plasmid vector (Fig. 23). The plasmid clone L was identified by restriction analysis and verified by nucleotide sequence analysis.

The immunoglobulin V gene was synthesized by P.T. Jones et al. (Jones, P.T., Dear, P.H., Foote, J., Neuberger, M.S. Winter, G., Nature 321: 522, (1986)) using oligonucleotides. It contains a PstI restriction cleavage site in the 5' region of the clone and is clored as HindIII/BamHI fragment in an M13 mp8 vector whose PstI cleavage site had been destroyed by cleavage, removal of the protruding ends and religation (Fig. 24).

II B) <u>Preparation of the mAb C gene portion:</u>

Example 16

A human IgG3 C gene was isolated from a human gene bank in EMBL3 phages (Frischauf et al., loc. cit. and Seeman et al., loc. cit.) and subcloned into the plasmid vector pUC 19 as a HindIII/SphI fragment 3.1 kb in size (clone 54.1.24) (Fig. 2).

The plasmid clone 54.1.24 was cleaved with HindII and Asp718, the protruding ends of the Asp718 cleavage site were removed with T_4 polymerase and religated with T_4 ligase. Restriction analysis and nucleic acid sequence determination were used to identify the clone 54.1.24 Delta Pol which, apart from SphI, PstI, SstI and EcoRI, no longer contains any restriction cleavage sites 3' of the human IgG3 C gene (Fig. 25).

The plasmid clone 54.1.24 Delta Pol was digested with BglII and SphI. The protruding ends were removed with T_4 polymerase and religated with T_4 ligase. Restriction analysis and nucleic acid sequence determination were used to identify the clone I which now contains only the CH_1 exon of the human IgG3 C gene (Fig. 26).

The plasmid clone I was cleaved with PstI, and the protruding ends were removed with T_4 polymerase. The B' insert which had been cut with XbaI and filled in with T_4 polymerase to give blunt ends was ligated into the resulting blunt ends. Restriction analysis and nucleic acid sequence determination were used to identify the clone K (Fig. 27) which contains a human IgG_3C_H1 exon and a 3' end of a HLA class I gene.

The plasmid clone K was cleaved with HindIII and EcoRI, the protruding ends were removed, and the insert was ligated in an SstI-cleaved pUC 19 plasmid whose ends had likewise been made blunt. The clone L which harbors the polylinker of the pUC 19 vector 5' from the C_H1 exon was

identified (Fig. 28).

The plasmid clone L was cleaved with EcoRI and HindIII, and the insert was purified and ligated into a HindIII- and EcoRI-cleaved KS$^+$ vector (Stratagene; Bluescript Exo/Mung DNA Sequencing System) whose PstI cleavage site had previously been destroyed by cleavage with PstI, T_4 polymerase treatment and religation. The clone M, from which it is possible to cut out the human $C_{\alpha}1$ exon with the HLA class I 3' end by a Bam HI cleavage, was identified (Fig. 29).

Example 17

Double-stranded DNA was prepared from the M13 mp8 clone V and cleaved with BamHI. The KS$^+$ clone M was cleaved with BamHI, and the insert M was purified. The M fragment was ligated into the BamHI-cleaved clone V, and nucleic acid sequence determination was used to identify the M13 clone N which contains an intact IgG3 gene (Fig. 30).

Double-stranded DNA was prepared from the M13 clone N and was cleaved with EcoRI, and the insert was purified. The plasmid clone D_1 was cleaved with EcoRI and ligated with the fragment N. The phage clone O in which the fragment N is cloned in the correct orientation into the clone D_1 was isolated (Fig. 31).

The plasmid clone O was subjected to a complete PstI cleavage and partial EcoRI cleavage and ligated with the linker fragment cut out of the plasmid vector L with EcoRI and PstI. The plasmid clone P contains the complete HLA B27w mAb fusion gene (Fig. 32, Fig. 33). This fusion gene can be expressed and secreted in human cells alone or in mouse cells together with the human beta$_2$ microglobulin gene if the expressing cells also contain an immunoglobulin light chain.

Key to Fig. 1

alpha 1, alpha 2 and alpha 3 denote the domains of the class I MHC antigen chain. The arrows point to the alpha helices which carry the allodeterminants. CM is meant to represent the cell membrane, and C the cell.

Key to Fig. 2 et seq.

EcoRI etc. represents the cleavage with the particular restriction endonuclease or represents the corresponding cleavage site. BamHI/Asp 718 denotes a restriction cleavage site destroyed by religation after filling-in.

TM denotes the transmembrane region.

3'NT denotes 3' non-translated
IgH p/E denotes the immunoglobulin heavy chain promoter/enhancer
* denotes: incomplete digestion
DS-DNA denotes: double-stranded DNA
SS-DNA denotes: single-stranded DNA

Key to Fig. 34:

s.CTL denotes: syngeneic cytotoxic T lymphocyte
TcR denotes: T-cell receptor
a.MHC class I denotes: allogenic MHC class I antigen
t.a.a. denotes: tumor-associated antigen
s.t.c. denotes: syngeneic tumor cell

CLAIMS

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. Antigenic constructs adapted to induce the specific activation of allospecific T cells and in which major histocompatibility complex (MHC) class I antigens are linked at the C- or N- terminal end to specific carrier molecules.

2. Antigenic constructs adapted to induce the specific activation of allospecific T cells and in which major histocompatibility complex (MHC) class I antigens are linked at the amino-terminal end to specific carrier molecules.

3. Antigenic constructs adapted to induce the specific activation of allospecific T cells and in which major histocompatibility complex (MHC) class I antigens are linked at the C-terminal end to specific carrier molecules.

4. Antigenic constructs as claimed in claim 1, 2 or 3, in which an MHC class I antigen in linked to one specific carrier molecule respectively.

5. Antigenic constructs as claimed in claim 1, 2, 3 or 4, in which the specific carrier molecules are CD4 domains.

6. Antigenic constructs as claimed in claim 1, 2, 3 or 4, in which the specific carrier molecules are monoclonal antibodies.

7. Antigenic constructs as claimed in claim 6, in which the monoclonal antibodies are shortened in the constant part of the heavy chain.

8. Antigenic constructs as claimed in claim 1, 2, 3, 4, 5, 6 or 7, in which the MHC class I antigen is HLA B27w or HLA B27k.

9. Antigenic constructs as claimed in claim 1, 2, 3, 4, 5, 6, 7 or 8, in which the MHC class I antigen is covalently bonded to the carrier molecule.

10. Antigenic constructs as claimed in claim 1, 2, 3, 4, 5, 6, 7 or 8, in which the MHC class I antigen is bonded

via avidin/biotin to the carrier molecule.

11. Antigenic constructs as claimed in claim 1, 2, 3, 4, 5, 6, 7 or 8, which are prepared by genetic manipulation by fusion of the DNAs coding for them.

12. A process for the preparation of antigenic constructs as claimed in claim 1, 2, 3, 4, 5, 6, 7, 8 or 9, which comprises the required parts of genes being fused in the form of their DNA, being provided with suitable regulation sequences and being expressed in suitable expression systems.

13. The use of the antigenic constructs as claimed in any one of claims 1 to 11 for the allogenization of target cells.

14. A pharmaceutical which contains antigenic constructs as claimed in any one of claims 1 to 11.

DATED this 26th day of July 1989
BEHRINGWERKE ARTIENGESELLSCHAFT

WATERMARK PATENT & TRADEMARK ATTORNEYS
50 QUEEN STREET
MELBOURNE. VIC. 3000.

DRAWINGS

FIG. 1

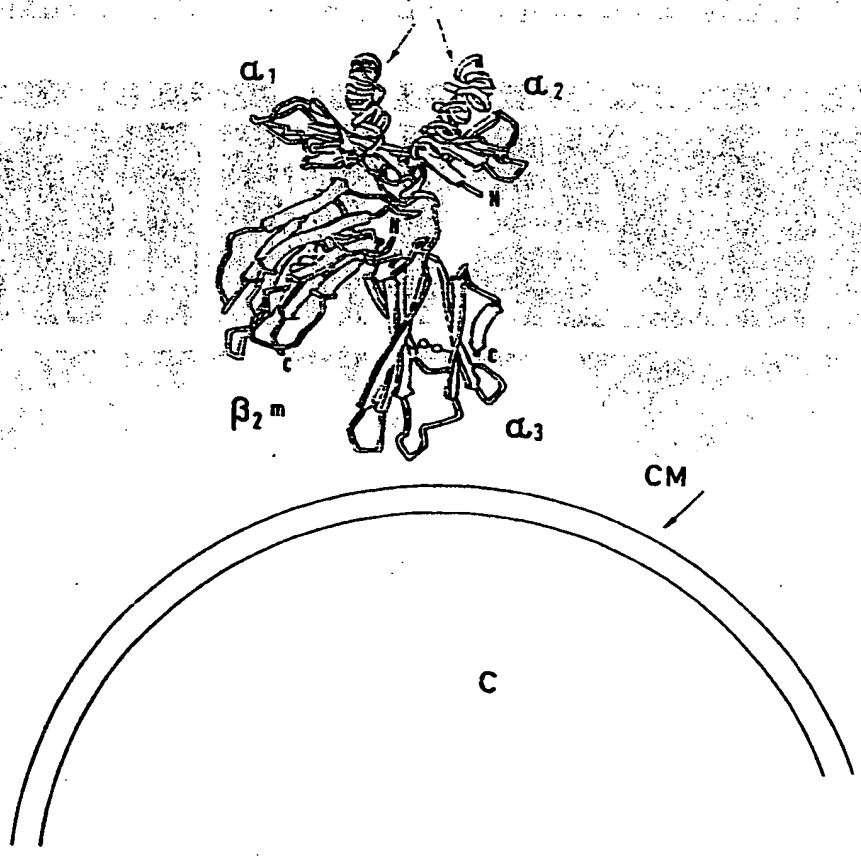
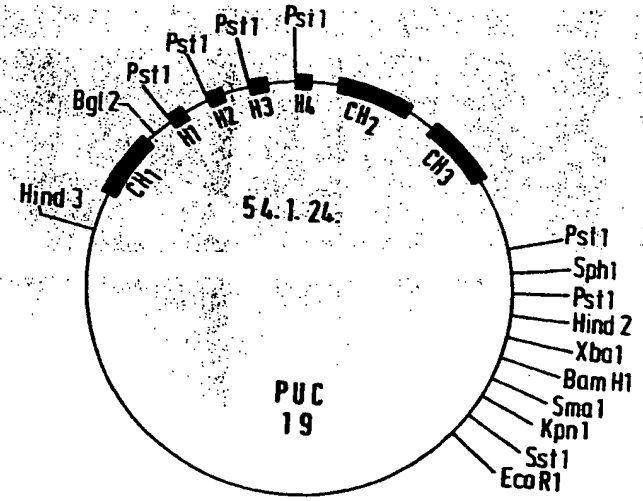


FIG. 2


Pst1
Pst1
Pst1
Pst1
Pst1
Bgl 2
H1
H2
H3
H4
CH2
CH3
CH1
Hind 3
54.1.24
PUC
19
Pst1
Sph1
Pst1
Hind 2
Xba1
Bam H1
Sma1
Kpn1
Sst1
EcoR1

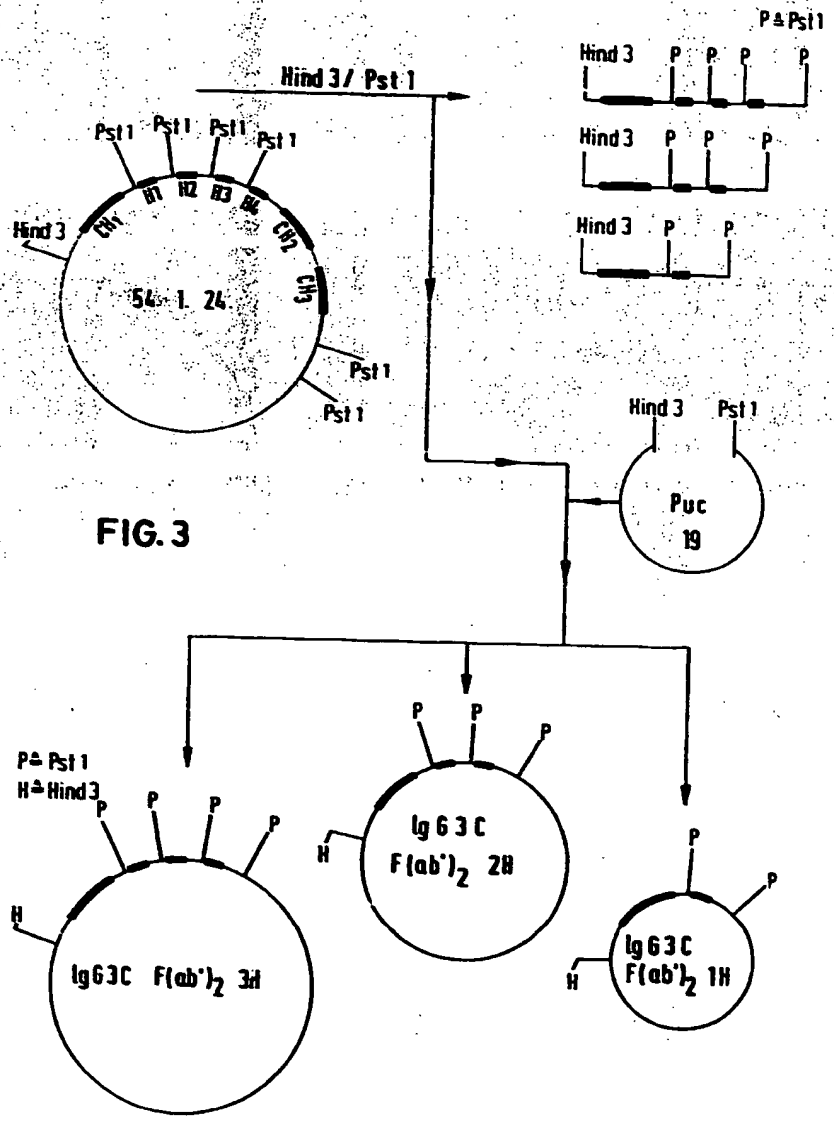
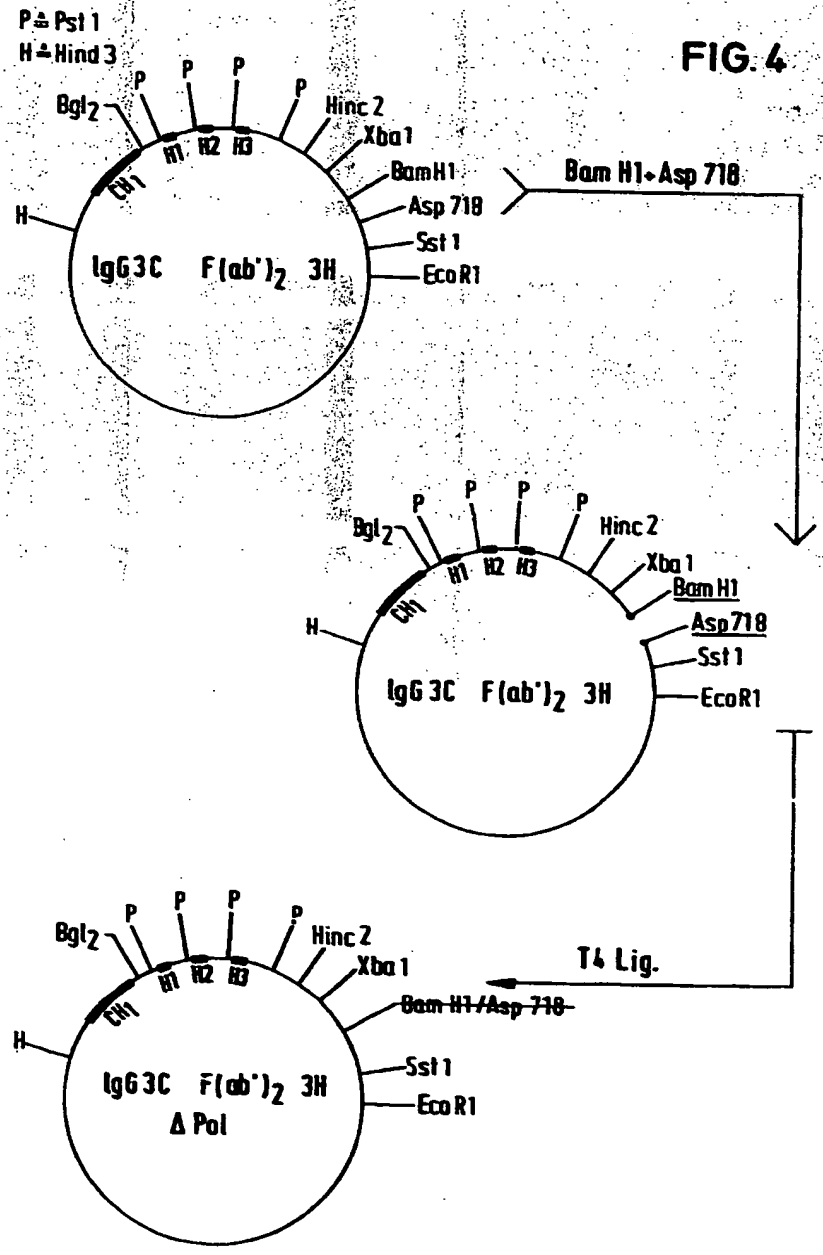

Hind 3 / Pst 1
P≙Pst 1
Hind 3 P P P P
Hind 3 P P P
Hind 3 P P
Pst 1 Pst 1 Pst 1 Pst 1
Hind 3
CH1 H1 H2 H3 HL CH2 CH3
54. 1. 24
Pst 1
Pst 1
Hind 3 Pst 1
Puc 19
P≙Pst 1
H≙Hind 3
P P P P
H
lg63C F(ab')2 3H
P P P
H
lg 63 C F(ab')2 2H
P P
H
lg 63 C F(ab')2 1H

FIG. 3

FIG. 4


P ≙ Pst 1
H ≙ Hind 3
Bgl2
P
P
P
P
Hinc 2
Xba 1
BamH1
Asp 718
Sst 1
EcoR1
H
CH1
H1
H2
H3
IgG3C F(ab')2 3H
Bam H1+Asp 718
IgG 3C F(ab')2 3H
T4 Lig.
Bam H1/Asp 718
IgG 3C F(ab')2 3H
Δ Pol

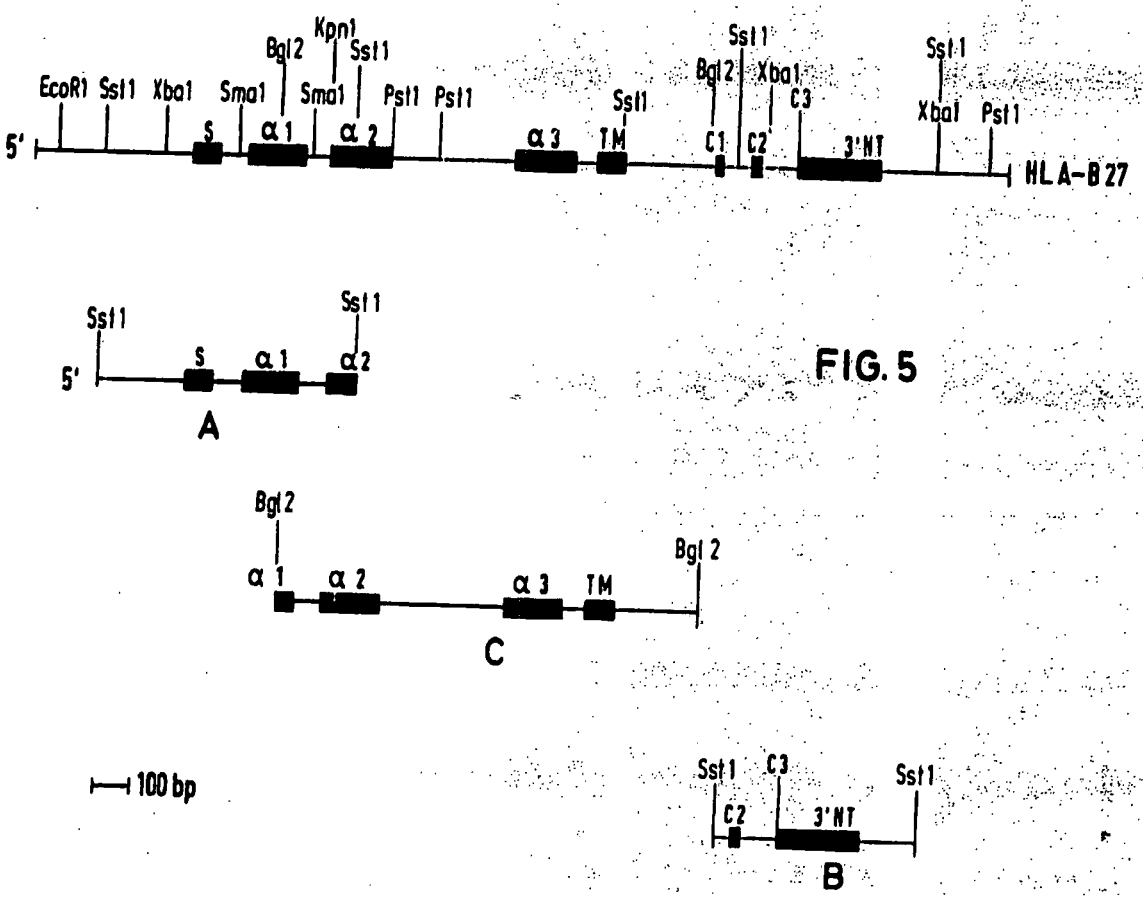

EcoR1
Sst1
Xba1
Sma1
Bgl2
Kpn1
Sma1
Sst1
Pst1
Pst1
Sst1
Sst1
Bgl2
Xba1
C3
Sst1
Xba1
Pst1
5'
S
α 1
α 2
α 3
TM
C1
C2
3'NT
HLA-B27
Sst1
Sst1
5'
S
α 1
α 2
A
Bgl2
α 1
α 2
α 3
TM
Bgl2
C
100 bp
Sst1
C3
Sst1
C2
3'NT
B

FIG. 5

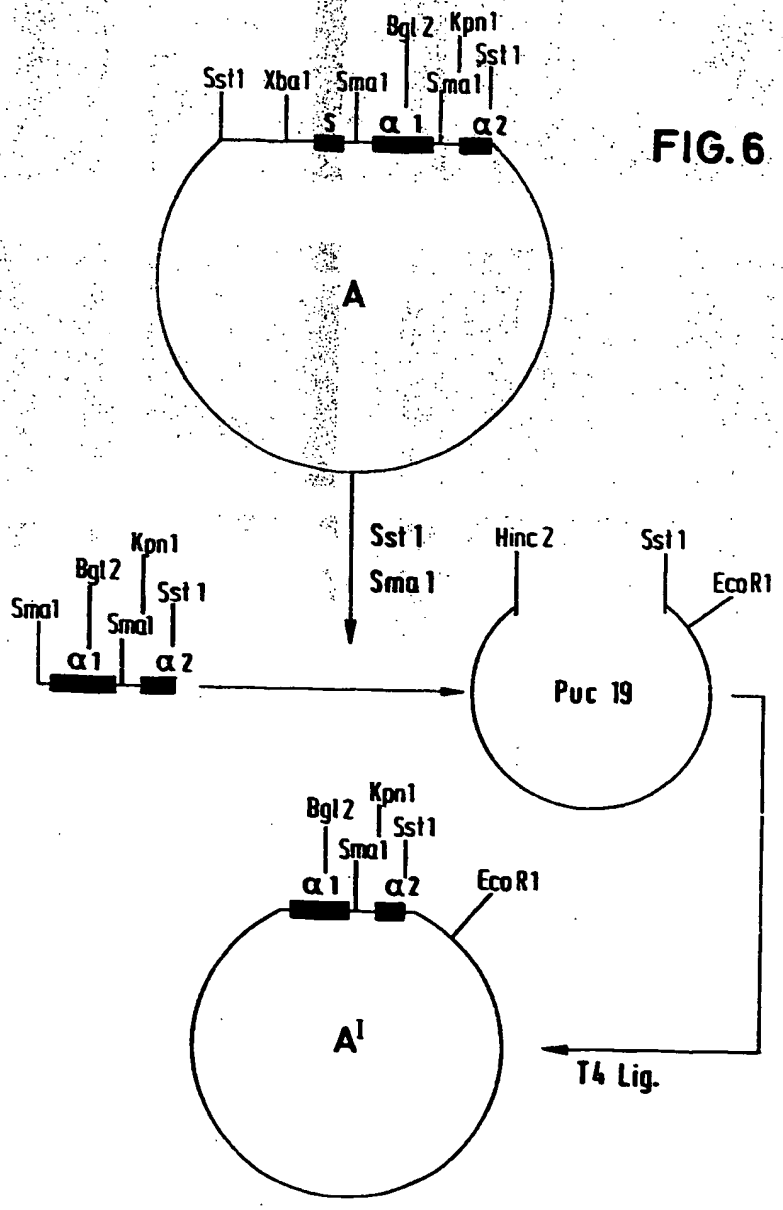

Sst1
Xba1
Sma1
Bgl 2
Kpn1
Sma1
Sst1
S
α 1
α2
A
Sst 1
Sma 1
Kpn1
Bgl2
Sst 1
Sma1
Sma1
α1
α2
Hinc 2
Sst 1
EcoR1
Puc 19
Bgl2
Kpn1
Sst1
Sma1
α1
α2
EcoR1
A^I
T4 Lig.

FIG. 6

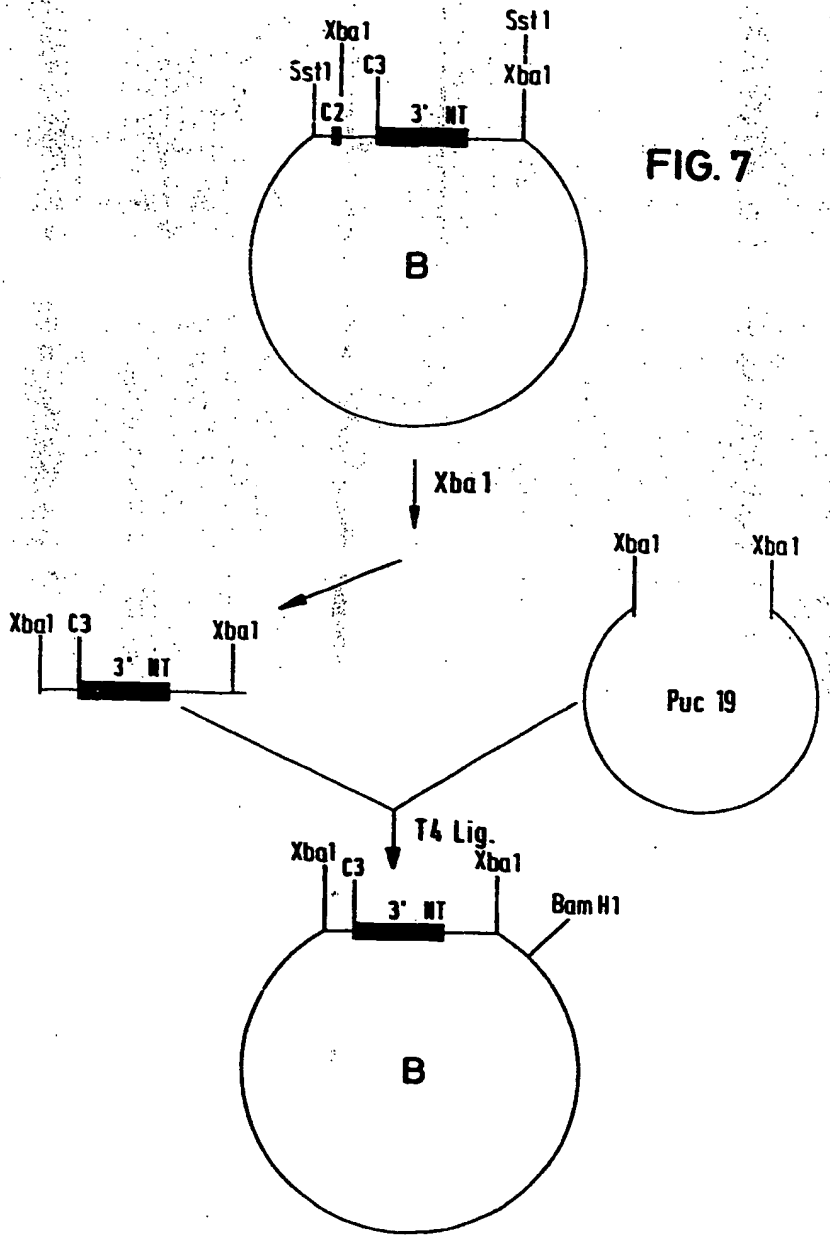

Xba1
Sst1
C3
Sst1
Xba1
C2
3' NT
B
Xba 1
Xba1
Xba1
Xba1 C3
Xba1
3' NT
Puc 19
T4 Lig.
Xba1
C3
Xba1
3' NT
BamH1
B

FIG. 7

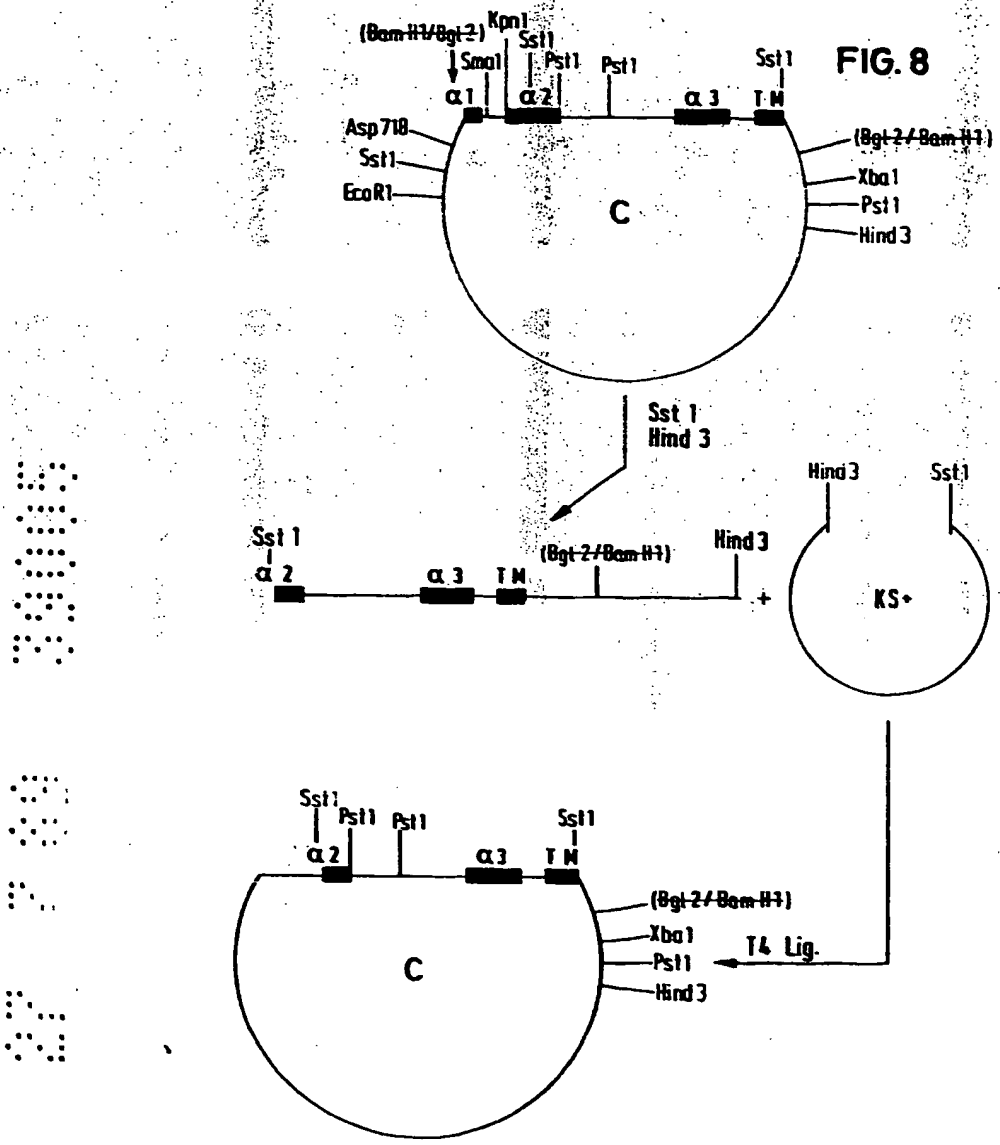

(Bam H1/Bgl 2)
Kpn1
Sst1
Sma1
Pst1
Pst1
Sst1
α 1
α 2
α 3
T M
Asp 718
Sst1
EcoR1
C
(Bgl 2/Bam H1)
Xba1
Pst1
Hind 3
Sst 1
Hind 3
Sst 1
α 2
α 3
T M
(Bgl 2/Bam H1)
Hind 3
+
Hind 3
Sst1
KS+
Sst1
Pst1
Pst1
Sst1
α 2
α 3
T M
(Bgl 2/Bam H1)
Xba1
Pst1
Hind 3
C
T4 Lig.

FIG. 8

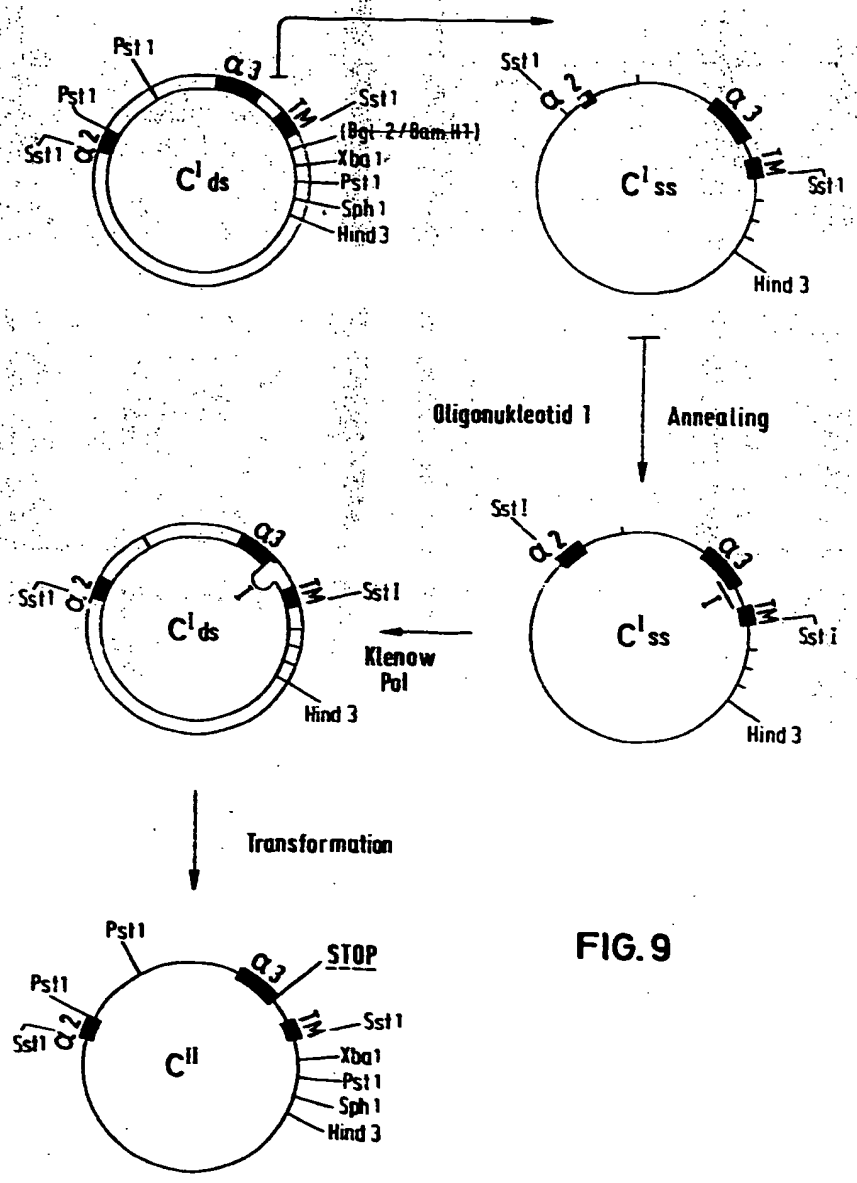
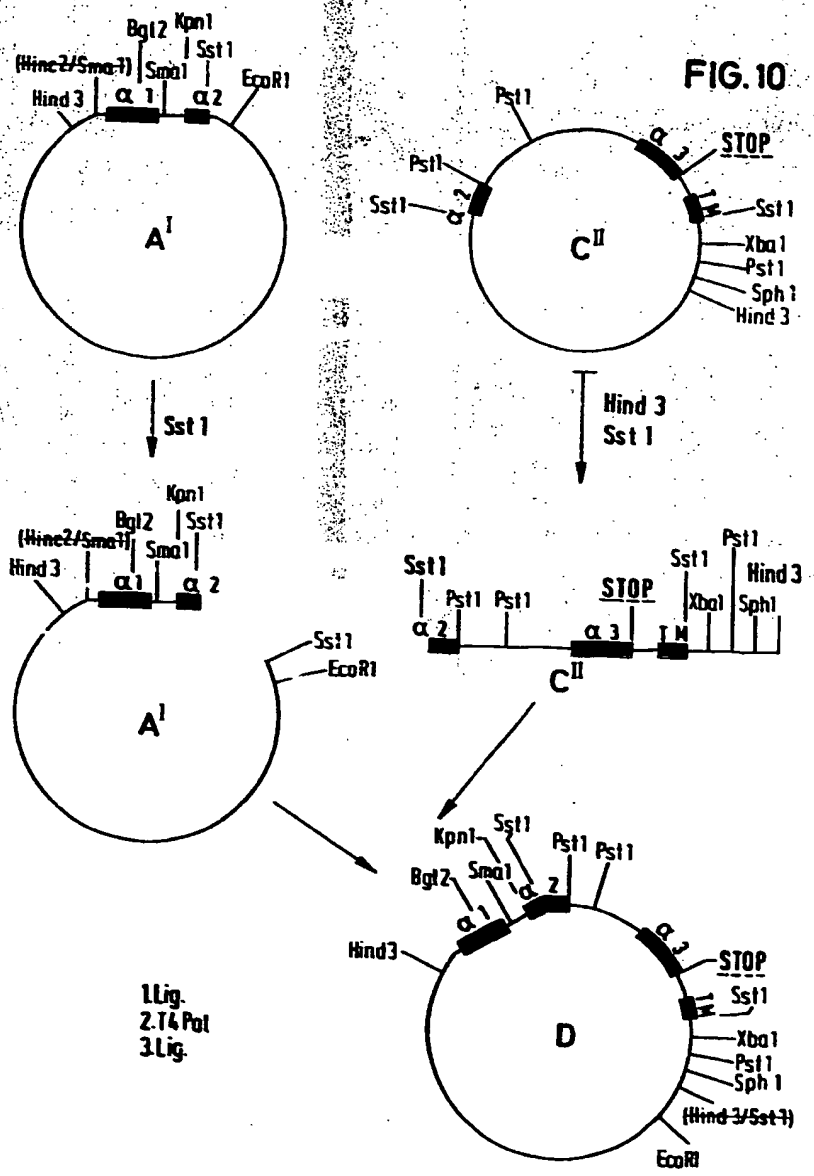

Pst 1
α 3
Pst 1
Sst 1
α 2
TM
Sst 1
(Bgl 2 / Bam H1)
Xba 1
Pst 1
Sph 1
Hind 3
C I ds
Sst 1
α 2
α 3
TM
Sst 1
C I ss
Hind 3
Oligonukleotid 1
Annealing
Sst 1
α 2
α 3
TM
Sst 1
C I ds
Hind 3
Klenow Pol
Sst 1
α 2
α 3
TM
Sst i
C I ss
Hind 3
Transformation
Pst 1
α 3
STOP
Pst 1
Sst 1
α 2
TM
Sst 1
C II
Xba 1
Pst 1
Sph 1
Hind 3

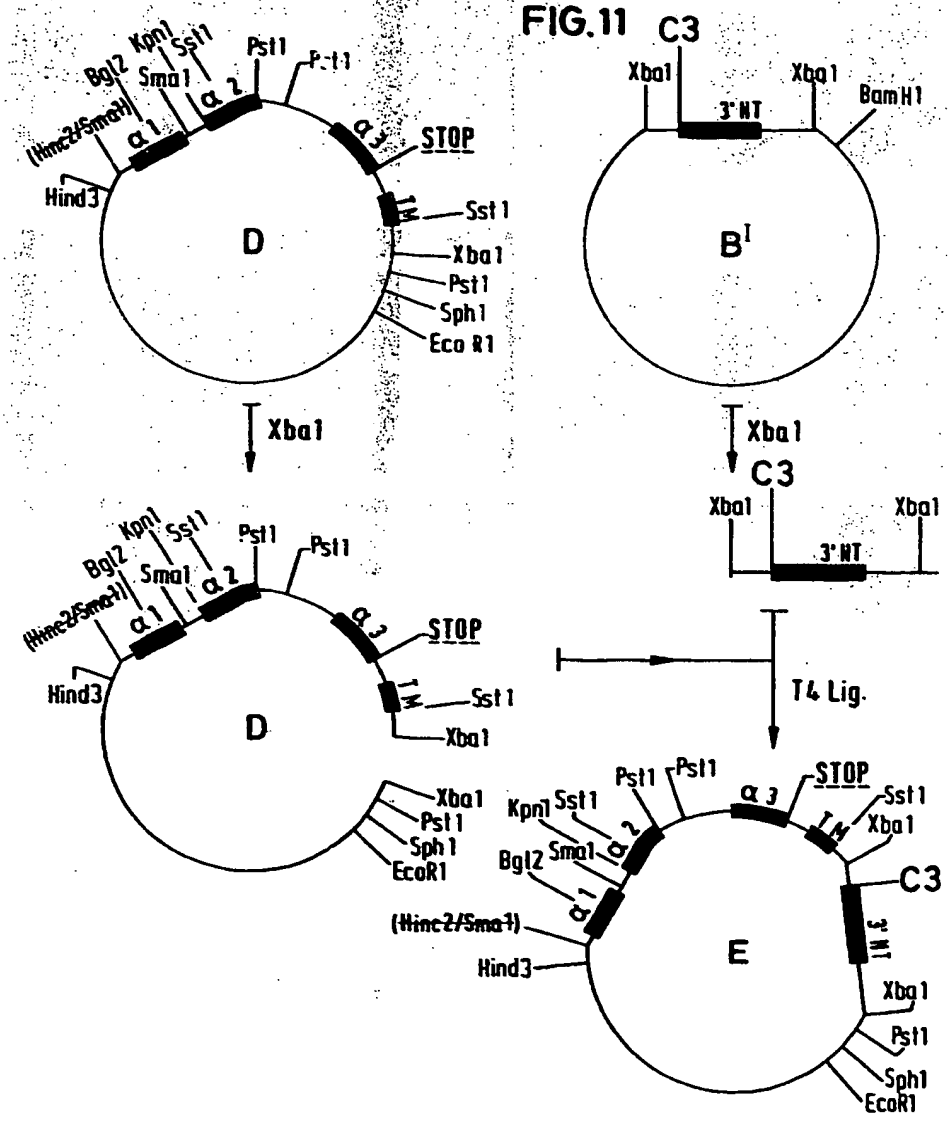
FIG. 9

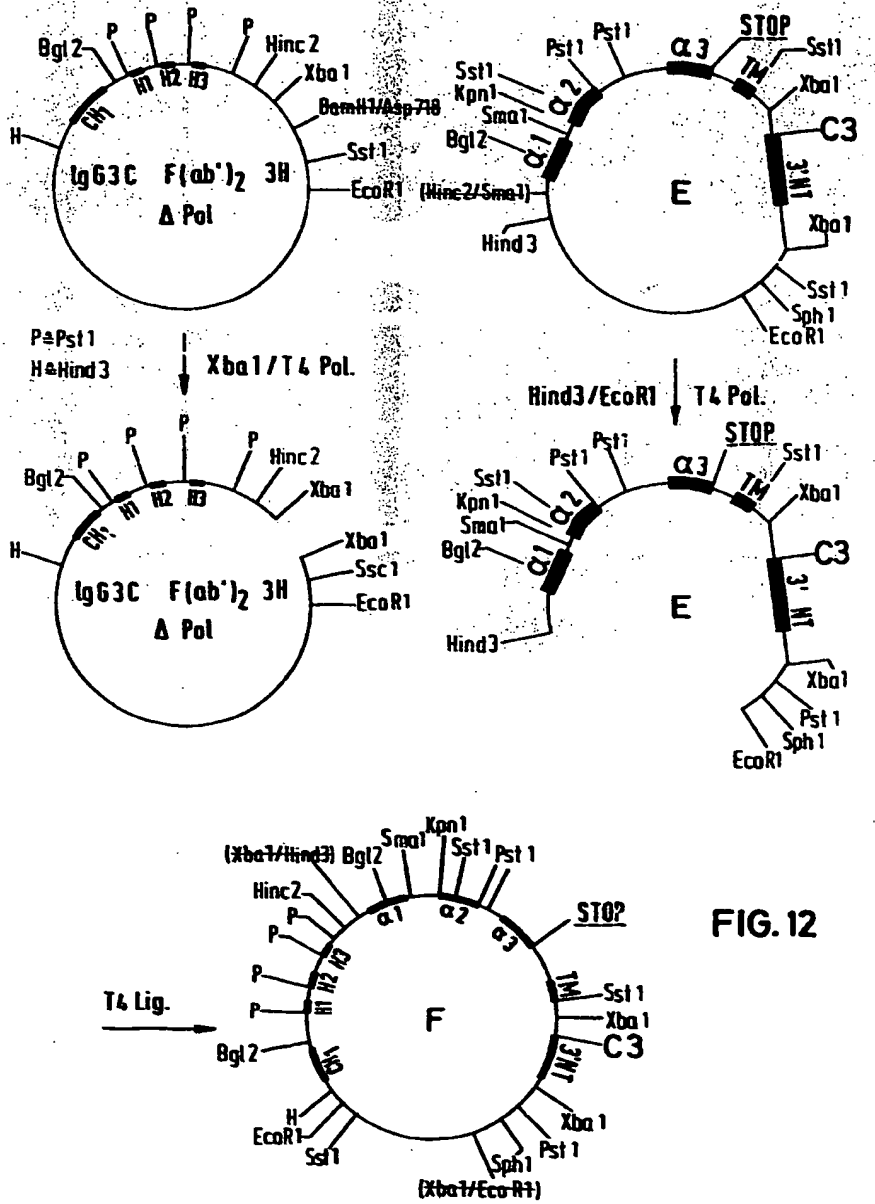
FIG. 10



1 Lig.
2. T4 Pol
3 Lig.

FIG.11


Kpn1
Sst1
Pst1
Pst1
Bgl2
Sma1
α 2
(Hinc2/Sma1)
α 1
α 3
STOP
Hind3
T4
Sst1
D
Xba1
Pst1
Sph1
Eco R1
Xba1
C3
Xba1
3'NT
Xba1
BamH1
B'
Xba1
C3
Xba1
Xba1
3'NT
T4 Lig.
Xba1
Pst1
Sph1
EcoR1
Kpn1
Sst1
Pst1
Pst1
STOP
Sst1
Bgl2
Sma1
α 2
α 3
T4
Xba1
C3
α 1
(Hinc2/Sma1)
E
3'NT
Hind3
Xba1
Pst1
Sph1
EcoR1


Bgl2
P
Hinc2
Xba1
BamH1/Asp718
Sst1
EcoR1
H
CH1
H1 H2 H3
IgG3C F(ab')2 3H
Δ Pol
P=Pst1
H=Hind3
Xba1/T4 Pol.
Ssc1
Pst1
Sst1
Kpn1
Sma1
α1
α2
α3
STOP
TM
C3
3'NT
(Hinc2/Sma1)
Hind3
Xba1
Sph1
EcoR1
E
Hind3/EcoR1
T4 Pol.

(Xba1/Hind3)
Bgl2
Smal
Kpn1
Sst1
Pst1
Hinc2
P
H1 H2 H3
α1
α2
α3
STOP
TM
C3
3'NT
H
EcoR1
Sst1
Sph1
(Xba1/EcoR1)
F
T4 Lig.

FIG. 12

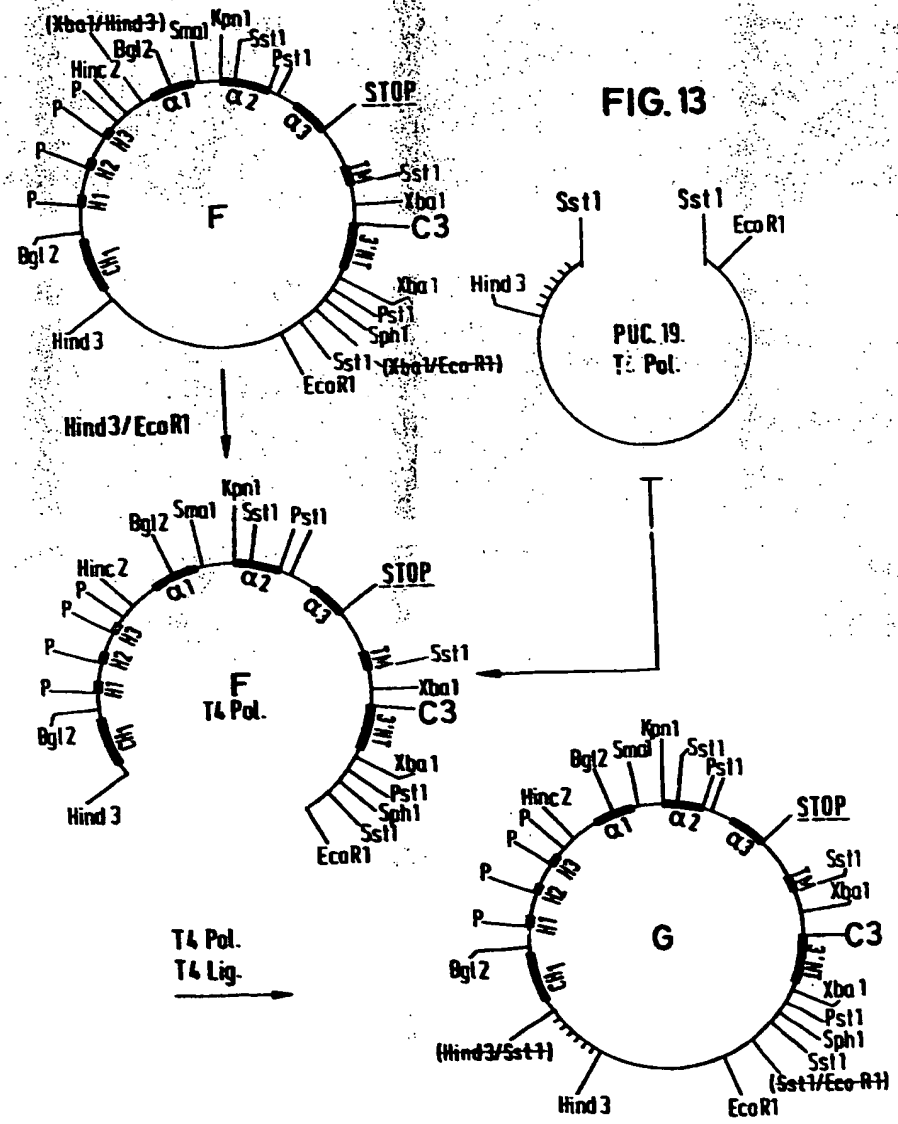

(Xba1/Hind3)
Sma1
Kpn1
Sst1
Pst1
Bgl2
Hinc2
P
STOP
α1
α2
α3
H1
H2
H3
F
Sst1
Xba1
C3
J.M.C
CH1
Bgl2
Hind3
Xba1
Pst1
Sph1
Sst1
(Xba1/Eco R1)
EcoR1
Hind3/EcoR1
Sst1
Sst1
EcoR1
Hind3
PUC. 19.
T4 Pol.
F
T4 Pol.
T4 Pol.
T4 Lig.
G
(Hind3/Sst1)
(Sst1/Eco R1)

FIG. 13

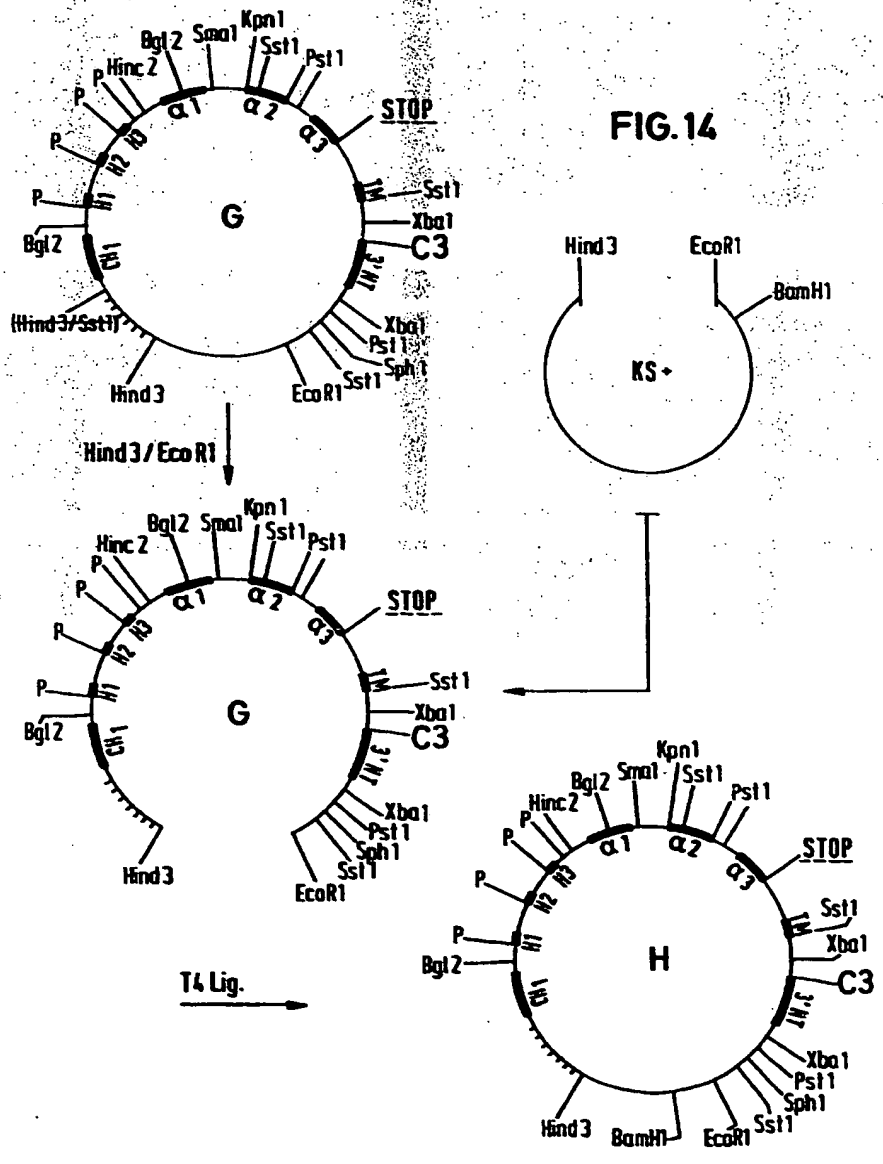

G
Bgl2
Hinc2
P
Smal
Kpn1
Sst1
Pst1
STOP
α1
α2
α3
H3
H2
H1
CH1
3'NT
C3
Xba1
Sph1
(Hind3/Sst1)
Hind3
EcoR1
Hind3/EcoR1
T4 Lig.
KS+
BamH1
H

FIG. 14

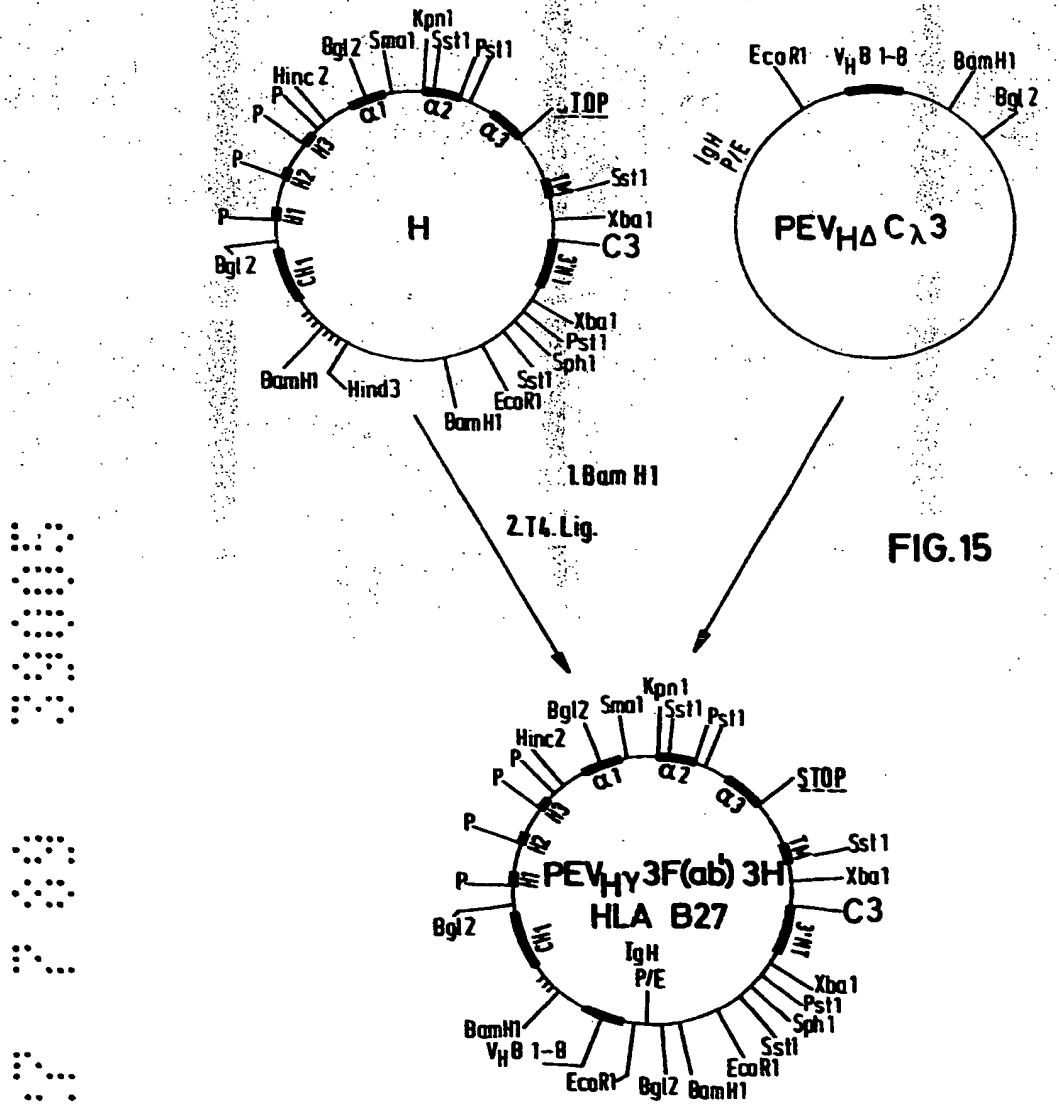

Kpn1
Bgl2
Sma1
Sst1
Pst1
Hinc 2
P
α1
α2
α3
STOP
H3
H2
H1
H
Sst1
Xba1
C3
CH1
Xba1
Pst1
Sph1
Sst1
EcoR1
BamH1
Hind3
BamH1
EcoR1
VHB 1-8
BamH1
Bgl 2
IgH
P/E
PEVHΔCλ3
1.Bam H1
2.T4.Lig.
PEVHγ3F(ab') 3H
HLA B27
IgH
P/E
BamH1
VHB 1-8
EcoR1
Bgl2
BamH1

FIG.15

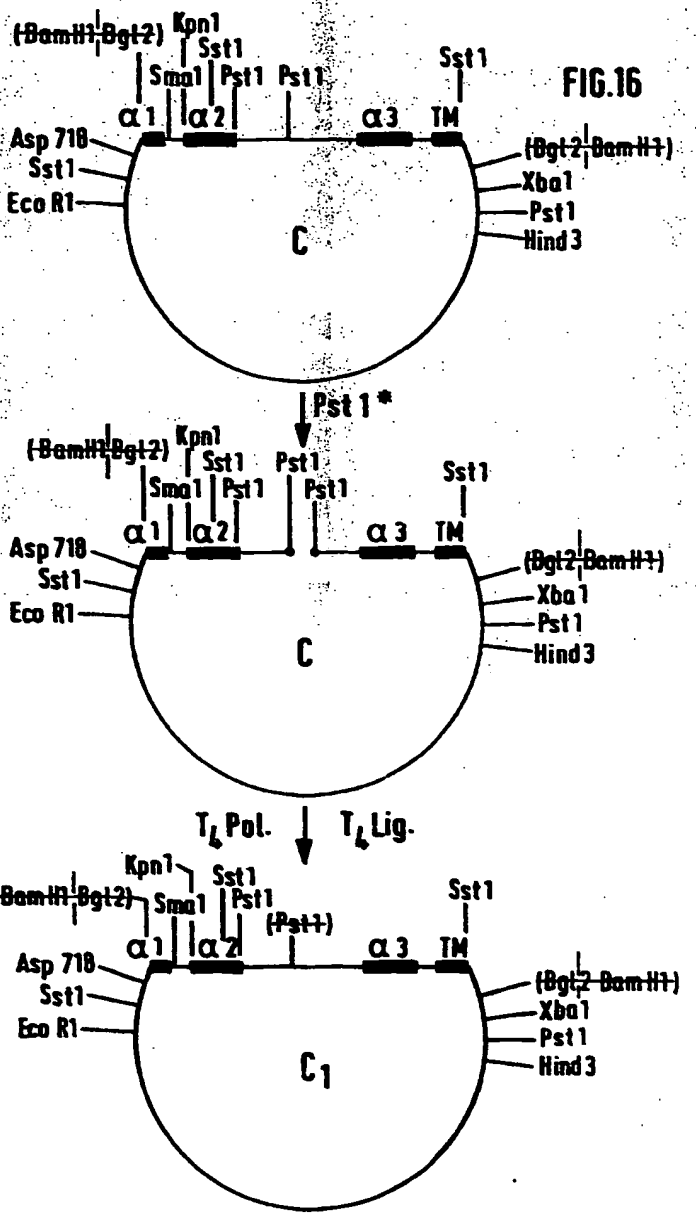

(BamH1 Bgl2)
Kpn1
Sst1
Sma1
Pst1
Pst1
Sst1
α1
α2
α3
TM
Asp 718
Sst1
Eco R1
(Bgl2 BamH1)
Xba1
Pst1
Hind3
C
Pst1 *
(BamH1 Bgl2)
Kpn1
Sst1
Pst1
Sma1
Pst1
Pst1
Sst1
α1
α2
α3
TM
Asp 718
Sst1
Eco R1
(Bgl2 BamH1)
Xba1
Pst1
Hind3
C
T4 Pol.
T4 Lig.
(BamH1 Bgl2)
Kpn1
Sst1
Sma1
Pst1
(Pst1)
Sst1
α1
α2
α3
TM
Asp 718
Sst1
Eco R1
(Bgl2 BamH1)
Xba1
Pst1
Hind3
C1

FIG.16

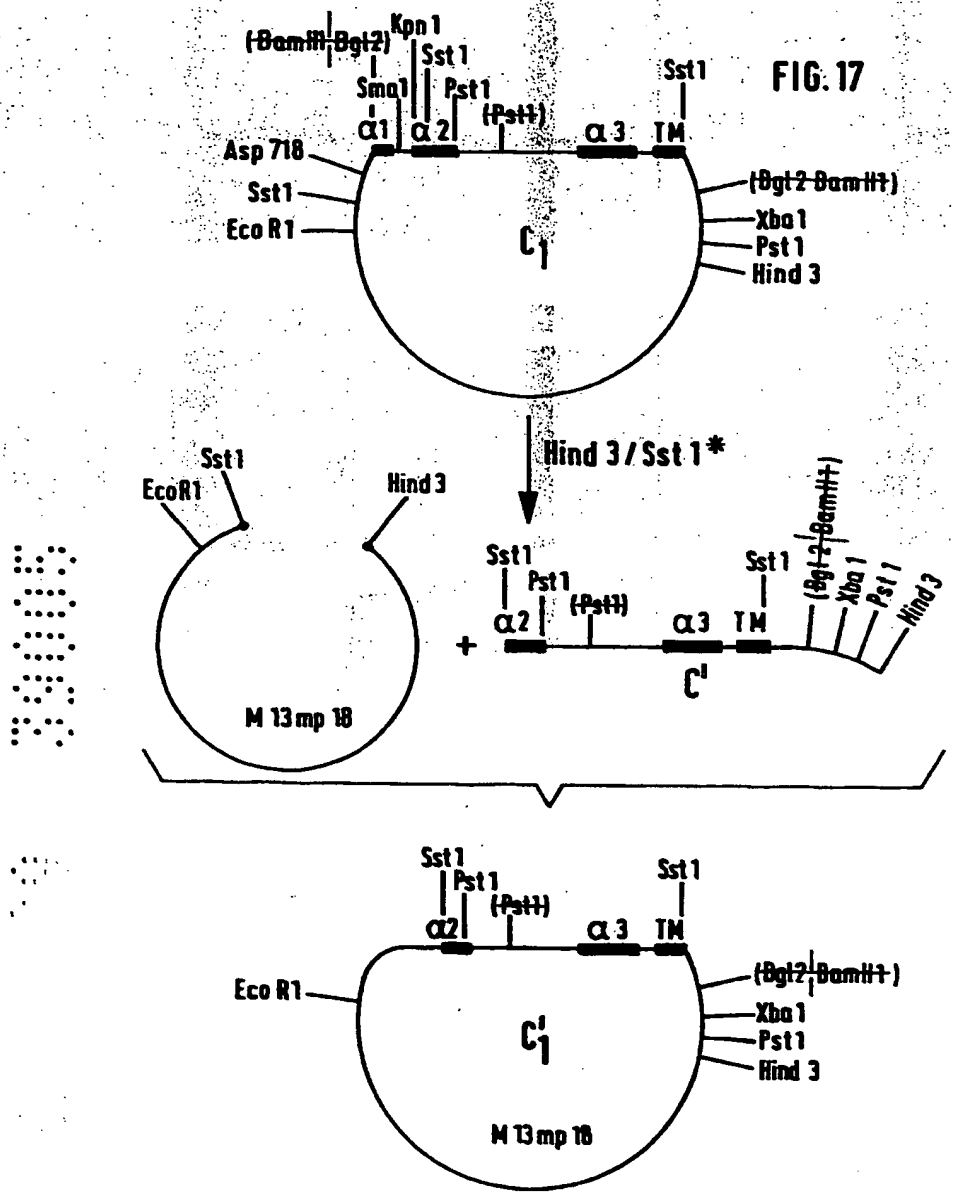

(BamH1 Bgl2)
Kpn 1
Sst 1
Sma1
Pst 1
(Pst1)
Sst1
α1
α 2
α 3
TM
Asp 718
Sst1
Eco R1
C1
(Bgl2 BamH1)
Xba 1
Pst 1
Hind 3
Hind 3 / Sst 1*
Sst1
EcoR1
Hind 3
M 13 mp 18
Sst1
Pst1
(Pst1)
α2
α 3
TM
Sst1
(Bgl2 BamH1)
Xba 1
Pst 1
Hind 3
C'
Sst 1
Pst 1
(Pst1)
α2
α 3
TM
Sst1
Eco R1
C'1
(Bgl2 BamH1)
Xba 1
Pst 1
Hind 3
M 13 mp 18

FIG. 17

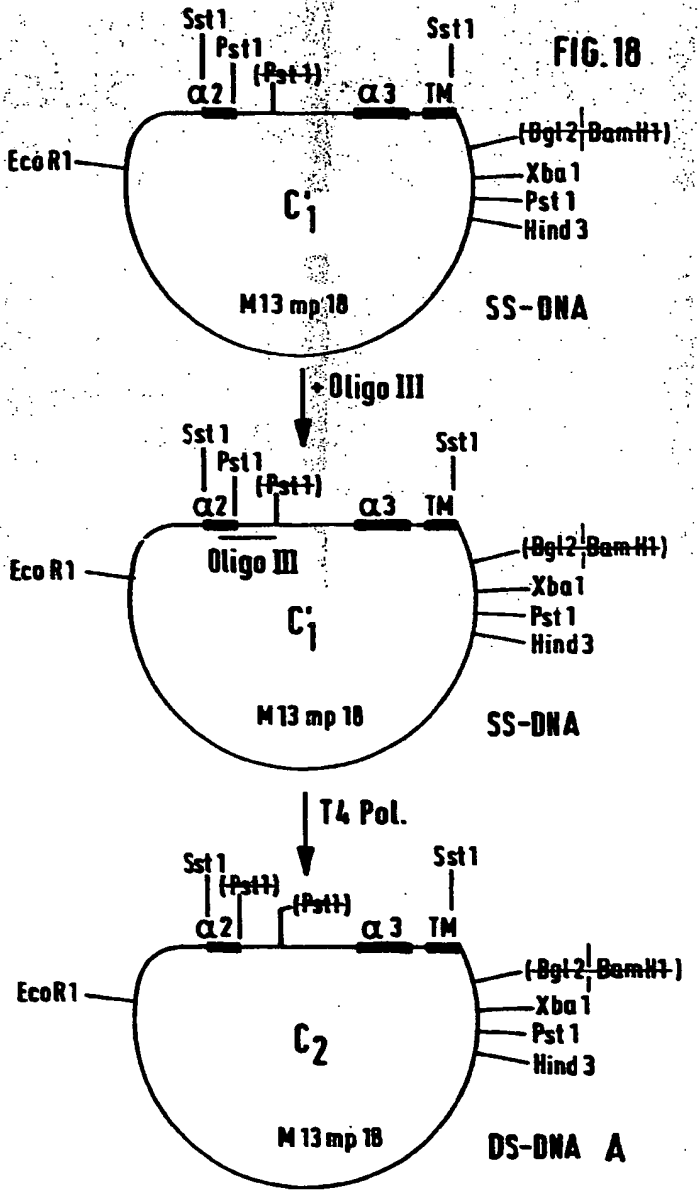

Sst1
Pst1
(Pst1)
α2
α3
TM
Sst1
(Bgl2 BamH1)
Xba1
Pst1
Hind3
EcoR1
C'1
M13 mp18
SS-DNA
+ Oligo III
Oligo III
T4 Pol.
C2
DS-DNA A

FIG. 18

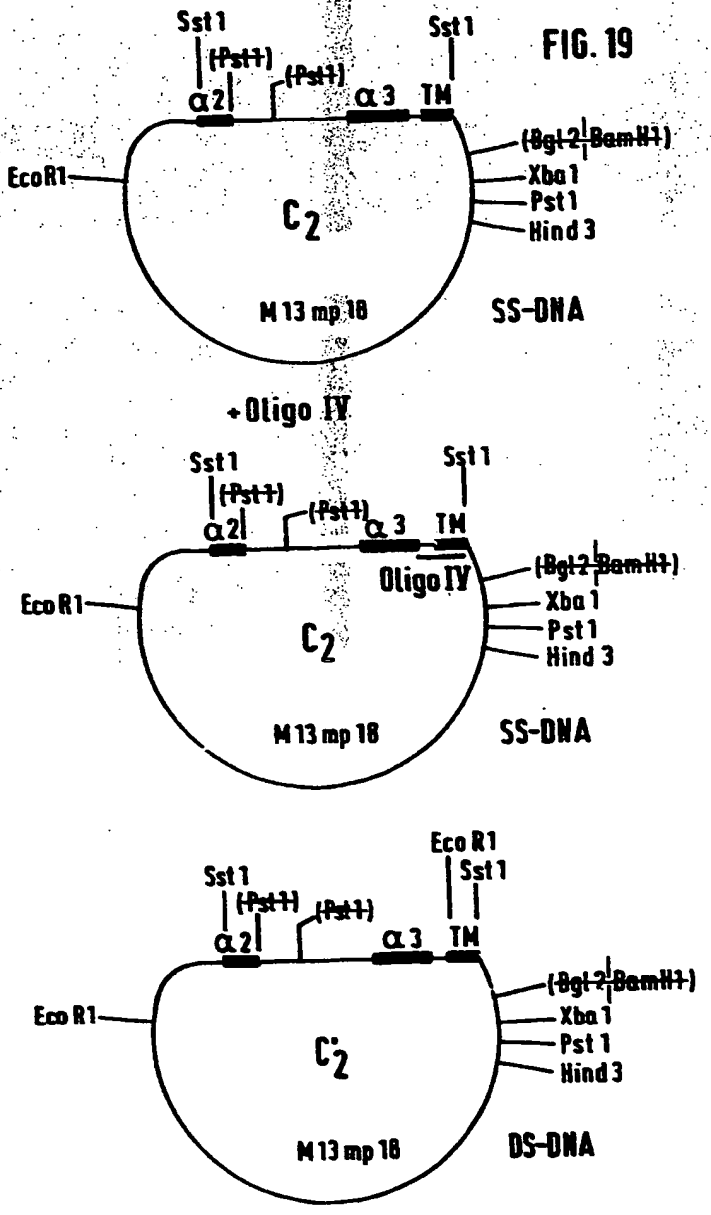

Sst 1
(Pst 1)
(Pst 1)
Sst 1
α 2
α 3
TM
(Bgl 2 | BamH 1)
Xba 1
Pst 1
Hind 3
EcoR1
C2
M 13 mp 18
SS-DNA
+ Oligo IV
Sst 1
(Pst 1)
(Pst 1)
Sst 1
α 2
α 3
TM
Oligo IV
(Bgl 2 | BamH 1)
Xba 1
Pst 1
Hind 3
Eco R1
C2
M 13 mp 18
SS-DNA
Eco R1
Sst 1
Sst 1
(Pst 1)
(Pst 1)
α 2
α 3
TM
(Bgl 2 | BamH 1)
Xba 1
Pst 1
Hind 3
Eco R1
C2
M 13 mp 18
DS-DNA

FIG. 19

FIG. 20

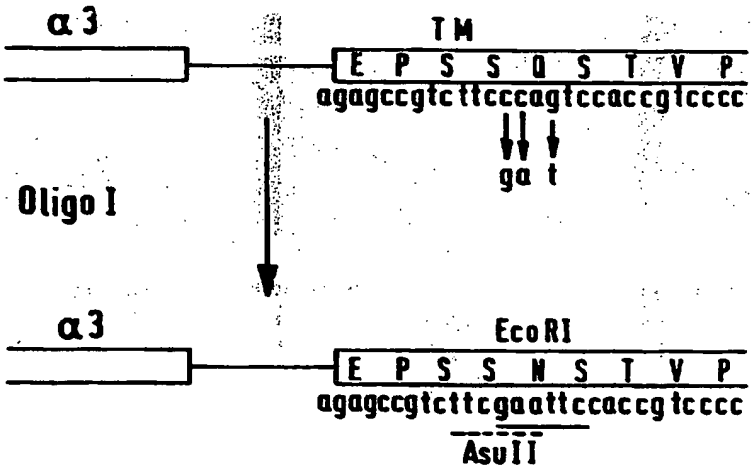

α3
TM
E P S S Q S T V P
agagccgtcttcccagtccaccgtcccc
ga t
Oligo I
α3
EcoRI
E P S S N S T V P
agagccgtcttcgaattccaccgtcccc
AsuII

FIG. 21

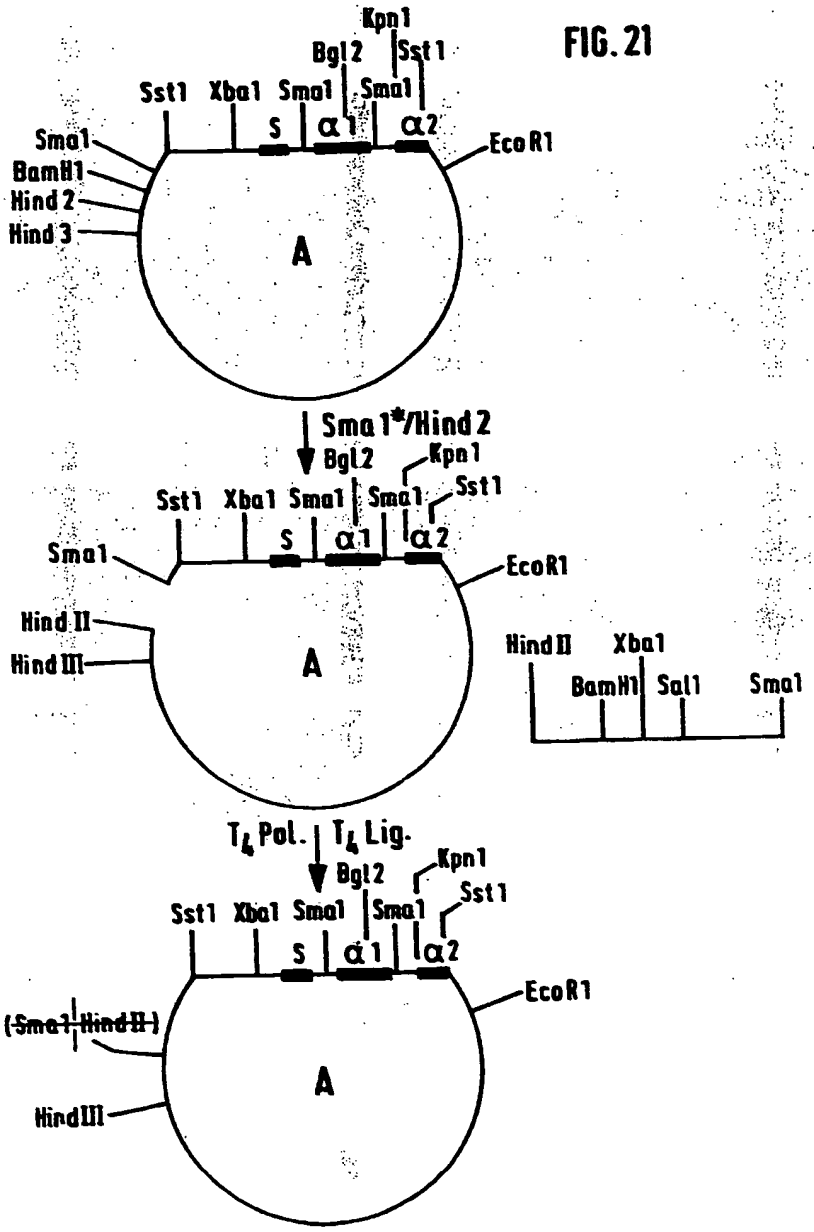
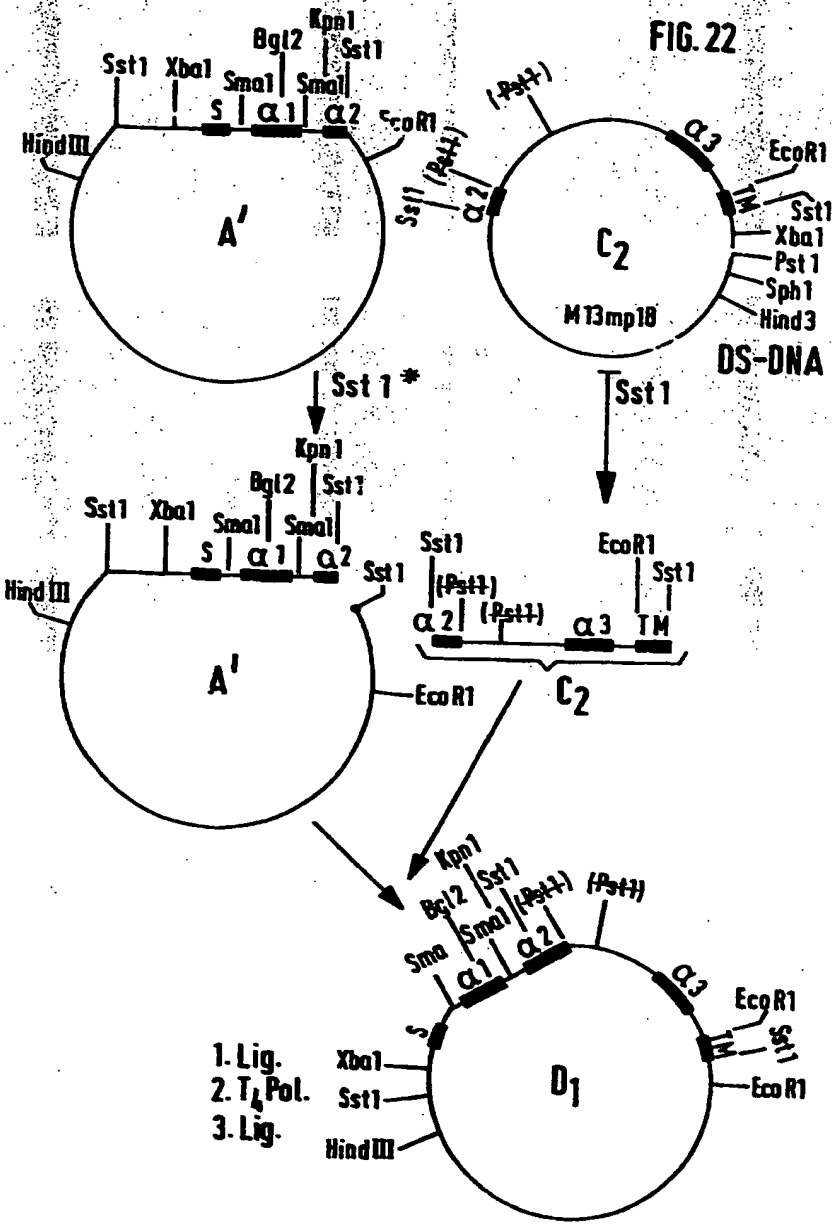

Kpn1
Bgl2
Sst1
Sst1
Xba1
Sma1
Sma1
S
α1
α2
Sma1
BamH1
Hind 2
Hind 3
EcoR1
A
Sma1*/Hind 2
Bgl2
Kpn1
Sst1
Sst1
Xba1
Sma1
Sma1
S
α1
α2
Sma1
EcoR1
Hind II
Hind III
A
Hind II
Xba1
BamH1
Sal1
Sma1
T4 Pol.
T4 Lig.
Bgl2
Kpn1
Sst1
Sst1
Xba1
Sma1
Sma1
S
α1
α2
EcoR1
(Sma1 Hind II)
Hind III
A

FIG. 22


Kpn1
Bgl2
Sst1
Sst1
Xba1
Sma1
Sma1
S
α1
α2
EcoR1
Hind III
A'
(Pst1)
Sst1
(Pst1)
α2
α3
EcoR1
TM
Sst1
Xba1
Pst1
Sph1
Hind3
C2
M13mp18
DS-DNA
Sst 1 *
Sst 1
Kpn1
Bgl2
Sst1
Sst1
Xba1
Sma1
Sma1
S
α1
α2
Sst1
Hind III
A'
EcoR1
Sst1
(Pst1)
α2
(Pst1)
α3
TM
EcoR1
Sst1
C2
Kpn1
Bgl2
Sst1
Sma1
(Pst1)
(Pst1)
Sma
α1
α2
α3
EcoR1
TM
Sst1
EcoR1
S
1. Lig.
2. T4 Pol.
3. Lig.
Xba1
Sst1
Hind III
D1

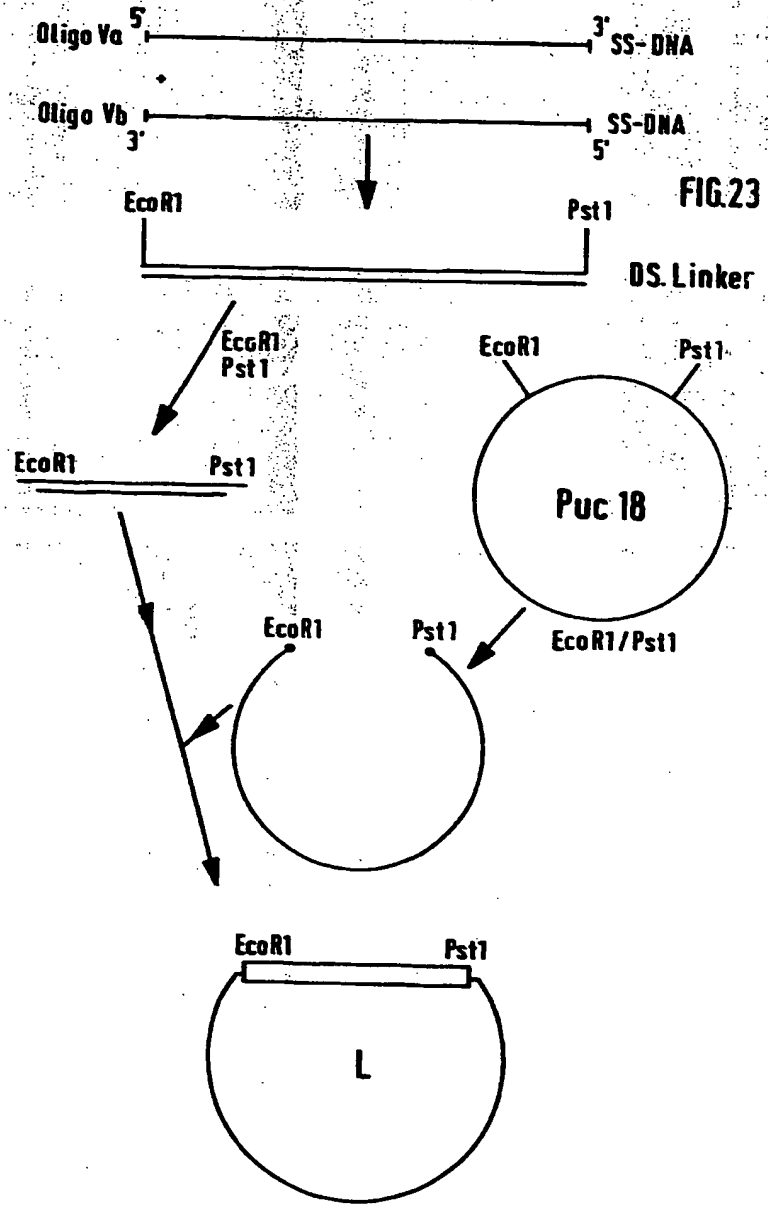

Oligo Va
5'
3'
SS-DNA
Oligo Vb
3'
SS-DNA
5'
EcoR1
Pst1
DS. Linker
EcoR1
Pst1
EcoR1
Pst1
EcoR1
Pst1
Puc 18
EcoR1
Pst1
EcoR1/Pst1
EcoR1
Pst1
L

FIG.23

FIG. 24

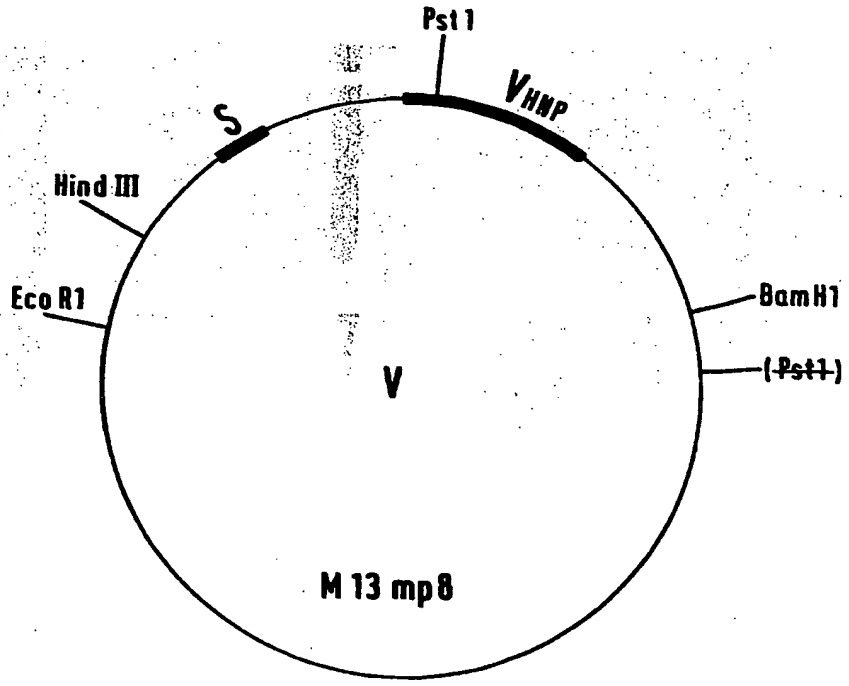


FIG. 25

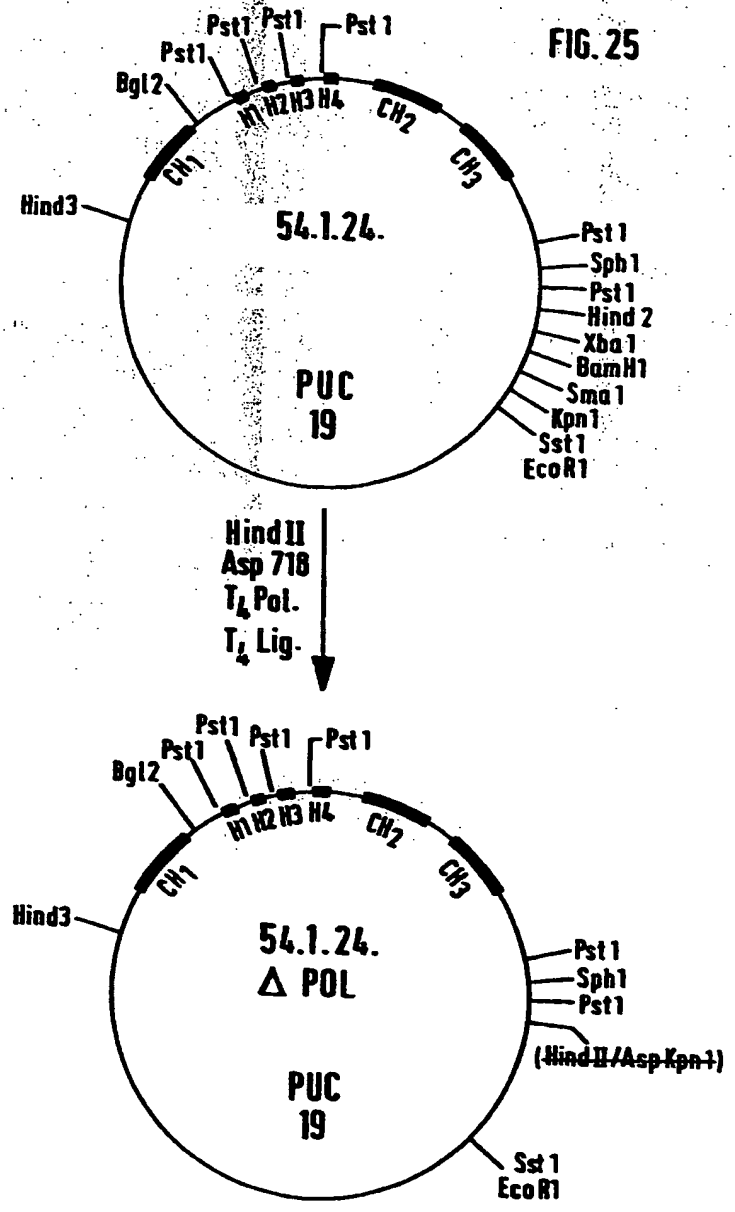

Pst1
Pst1
Pst1
Pst1
Pst 1
Bgl2
H1
H2
H3
H4
CH1
CH2
CH3
Hind3
54.1.24.
PUC
19
Pst 1
Sph1
Pst 1
Hind 2
Xba 1
BamH1
Sma 1
Kpn1
Sst1
EcoR1
Hind II
Asp 718
T4 Pol.
T4 Lig.
54.1.24.
Δ POL
(Hind II/Asp Kpn 1)

FIG.26

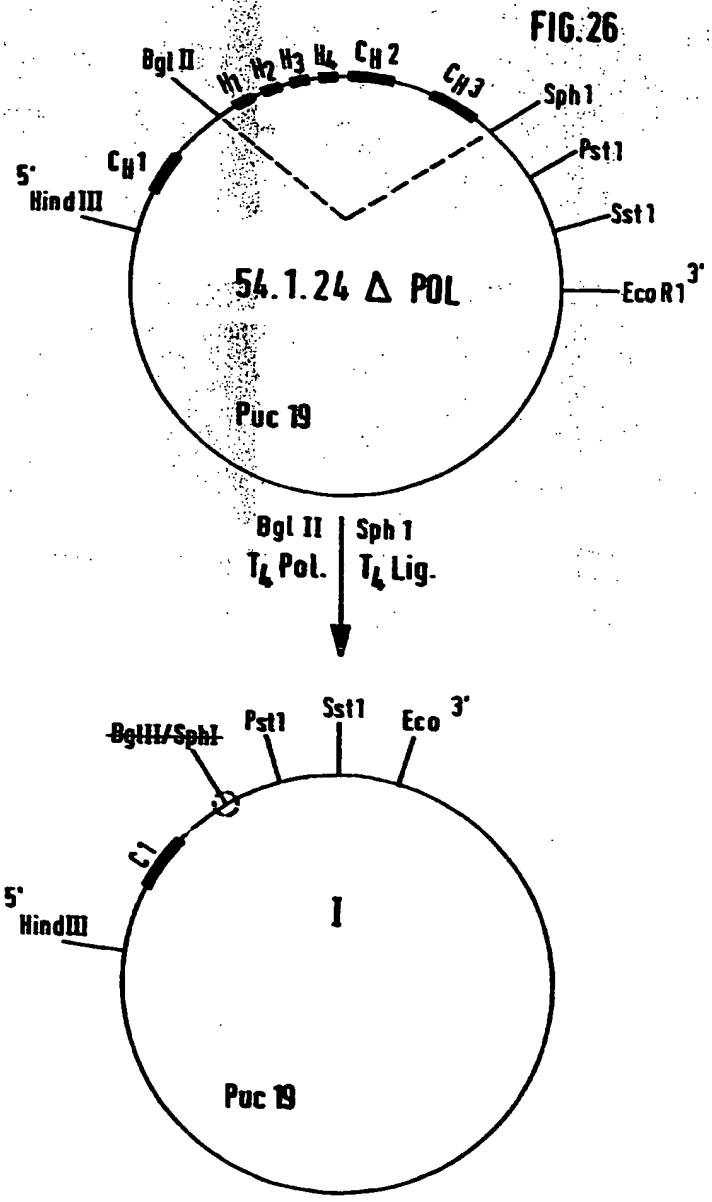

Bgl II
H1
H2
H3
H4
CH2
CH3
Sph1
Pst1
Sst1
EcoR1 3'
5'
HindIII
CH1
54.1.24 Δ POL
Puc 19
Bgl II
T4 Pol.
Sph 1
T4 Lig.
BglII/SphI
Pst1
Sst1
Eco 3'
C1
5'
HindIII
I
Puc 19

FIG. 27

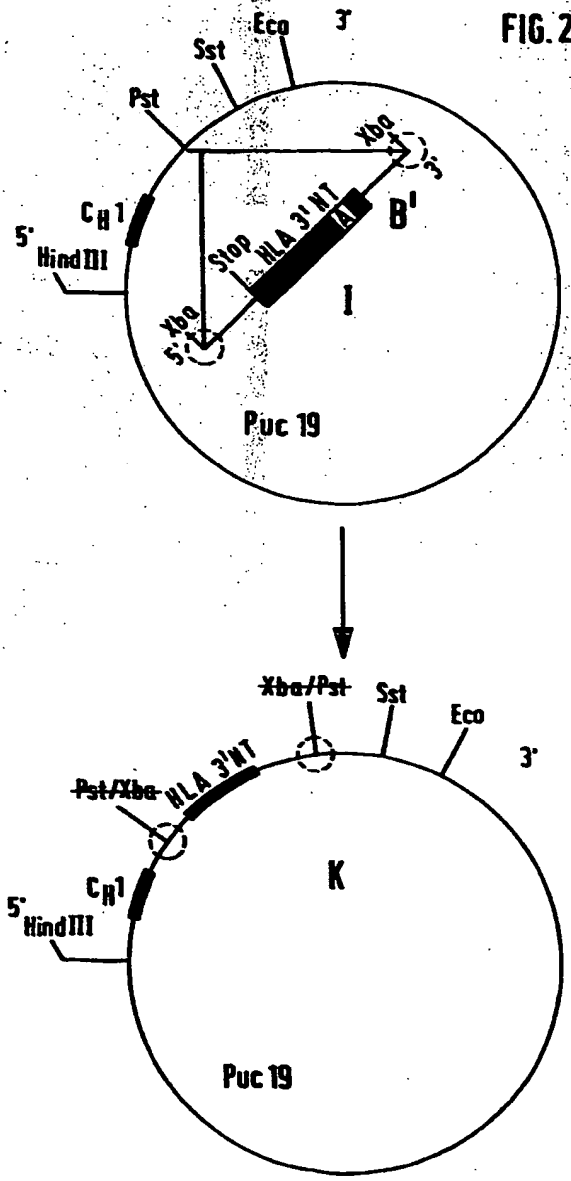

Eco
3'
Sst
Pst
Xba
3'
C_H1
5'
Hind III
Stop
HLA 3' NT
A1
B'
I
Xba
5'
Puc 19
Xba/Pst
Sst
Eco
3'
Pst/Xba
HLA 3'NT
K
C_H1
5'
Hind III
Puc 19

FIG. 28

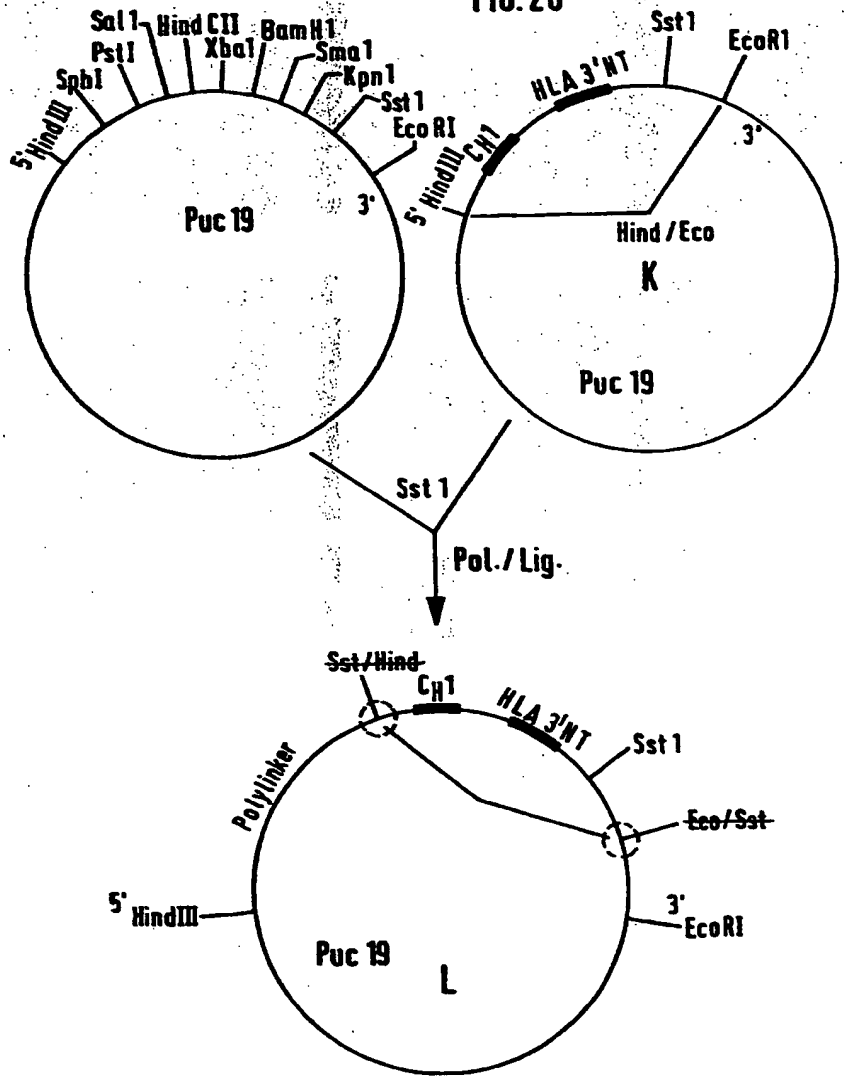

Sal 1
Hind CII
BamH1
Pst I
Xba I
Sma 1
Sph I
Kpn 1
5' HindIII
Sst 1
Eco RI
3'
Puc 19
Sst 1
EcoR1
HLA 3'NT
3'
5' HindIII
CH1
Hind / Eco
K
Puc 19
Sst 1
Pol. / Lig.
Sst / Hind
CH1
HLA 3'NT
Sst 1
Polylinker
Eco / Sst
5' HindIII
3'
EcoRI
Puc 19
L

FIG. 29

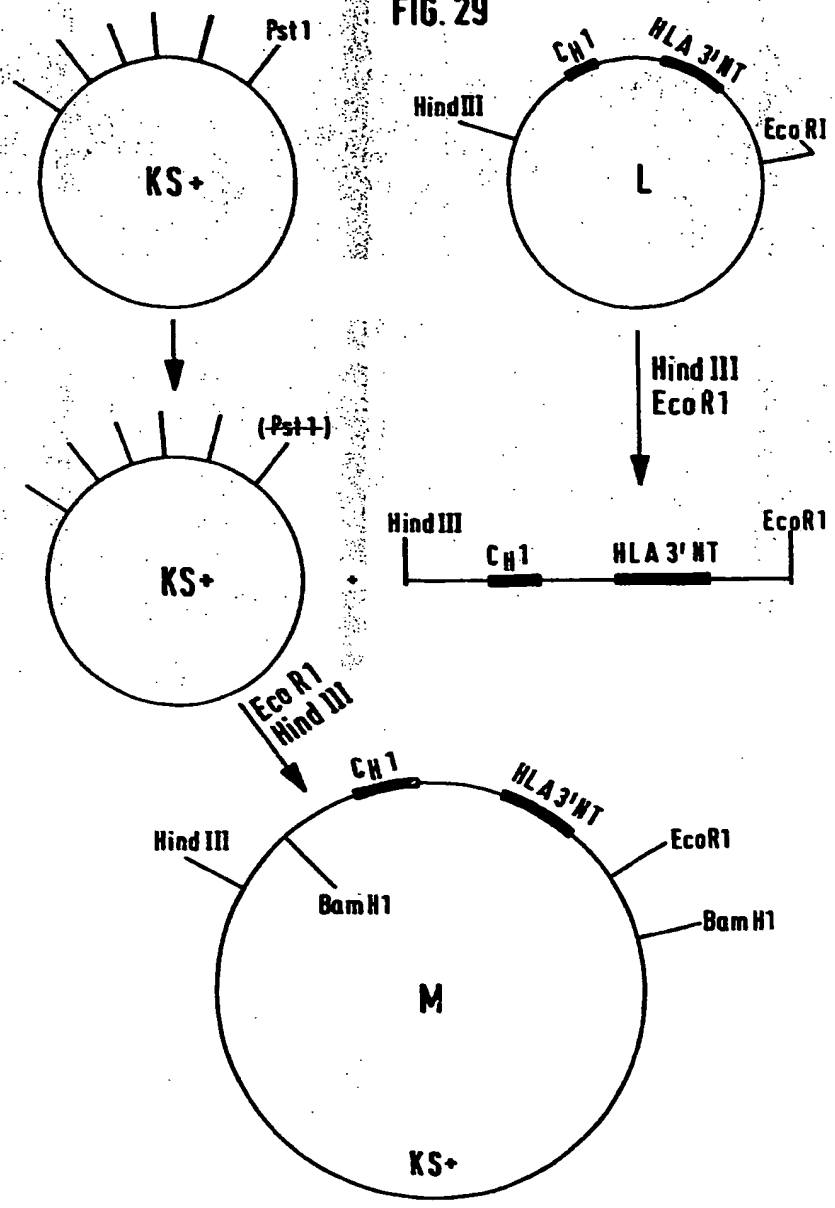

Pst1
KS+
(Pst1)
KS+
Eco R1
Hind III
CH1
HLA 3'NT
Hind III
Bam H1
EcoR1
Bam H1
M
KS+
CH1
HLA 3'NT
Hind III
EcoR1
L
Hind III
Eco R1
Hind III
CH1
HLA 3'NT
EcoR1

FIG.30

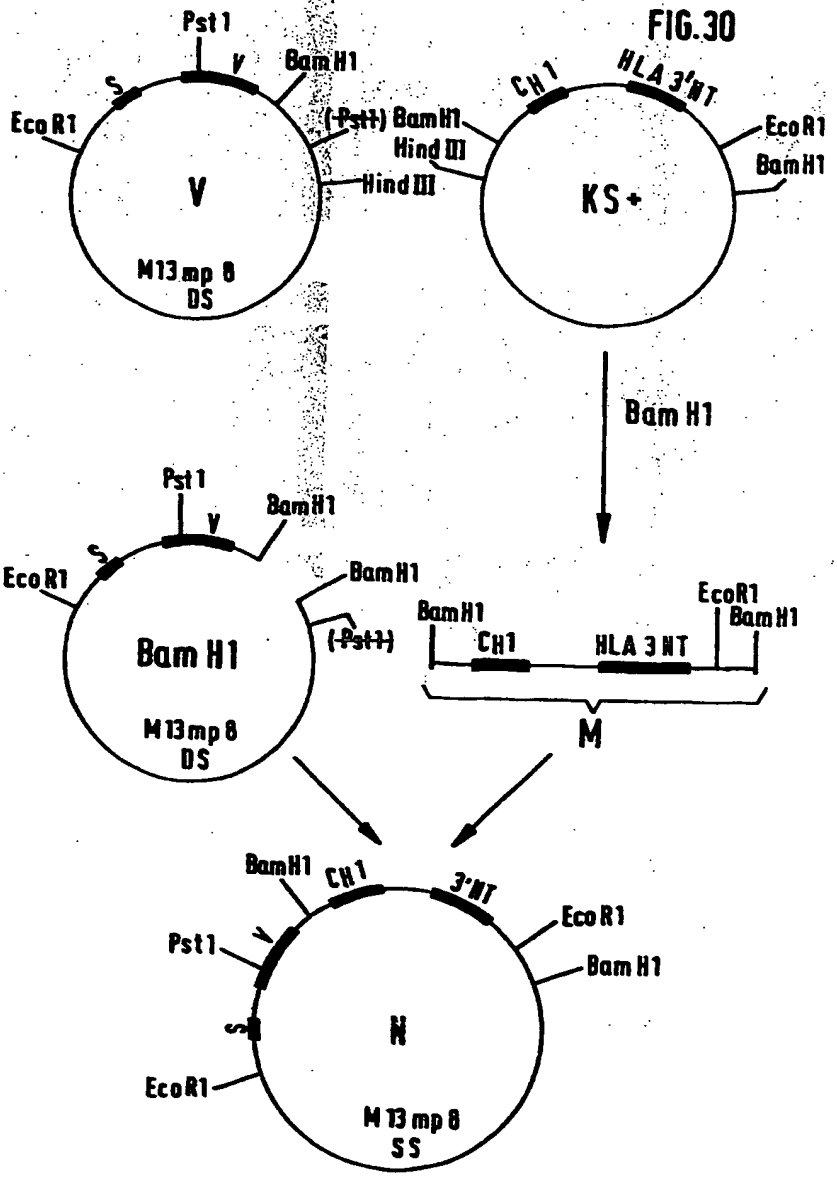

Pst 1
V
BamH1
S
EcoR1
(-Pst1)
BamH1
Hind III
Hind III
V
M13 mp 8
DS
CH1
HLA 3'NT
EcoR1
BamH1
KS+
Bam H1
Pst 1
V
BamH1
S
EcoR1
BamH1
(-Pst1)
Bam H1
M13 mp 8
DS
BamH1
CH1
HLA 3 NT
EcoR1
BamH1
M
BamH1
CH1
3'NT
EcoR1
Pst 1
V
Bam H1
S
N
EcoR1
M13 mp 8
SS

FIG. 31

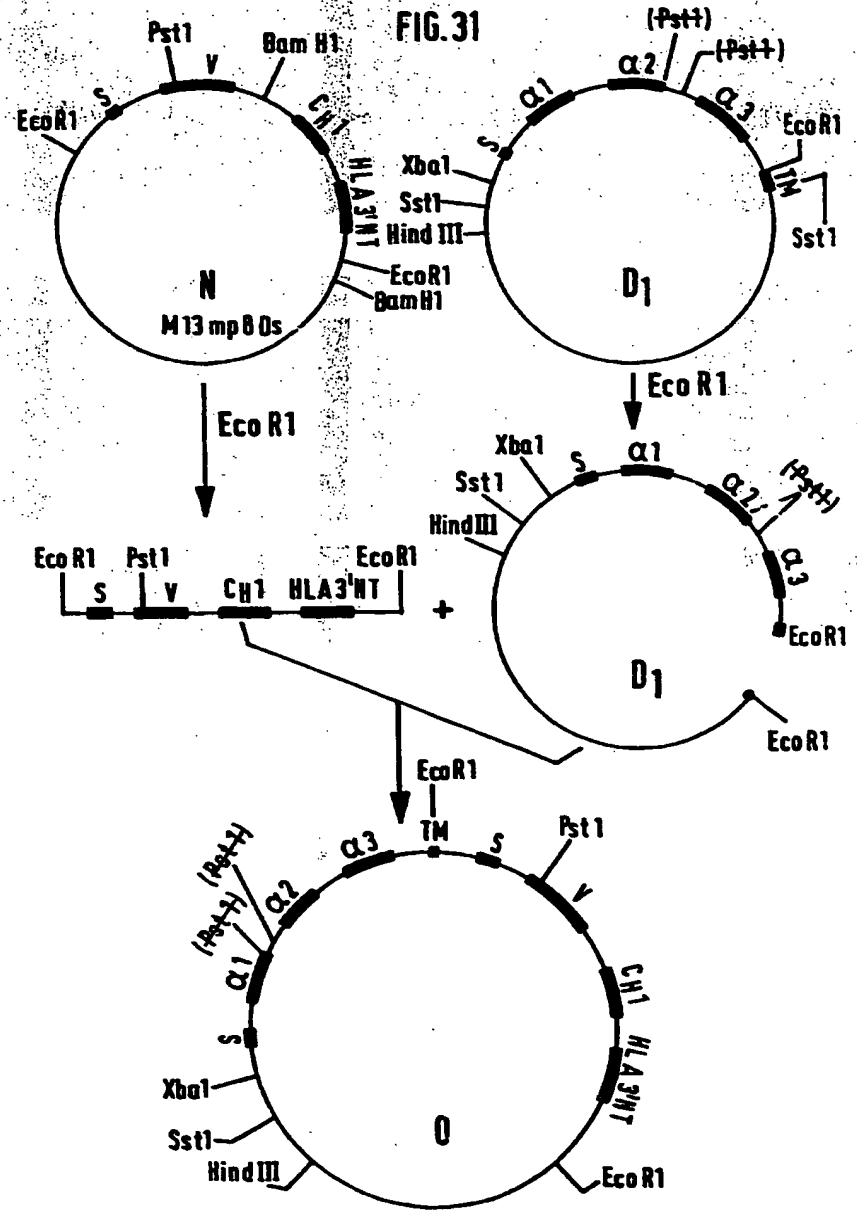

Pst1
V
Bam H1
EcoR1
S
CH1
HLA3'NT
N
M13 mp8 Os
EcoR1
BamH1
(Pst1)
(Pst1)
α2
α1
α3
EcoR1
S
Xba1
Sst1
Hind III
TM
Sst1
D1
Eco R1
Eco R1
Xba1
S
α1
Sst1
Hind III
α2
(Pst1)
α3
EcoR1
D1
EcoR1
EcoR1
Pst1
S
V
CH1
HLA3'NT
EcoR1
+
EcoR1
TM
α3
S
Pst1
V
(Pst1)
(Pst1)
α2
α1
S
CH1
HLA3'NT
Xba1
Sst1
Hind III
O
EcoR1

FIG. 32

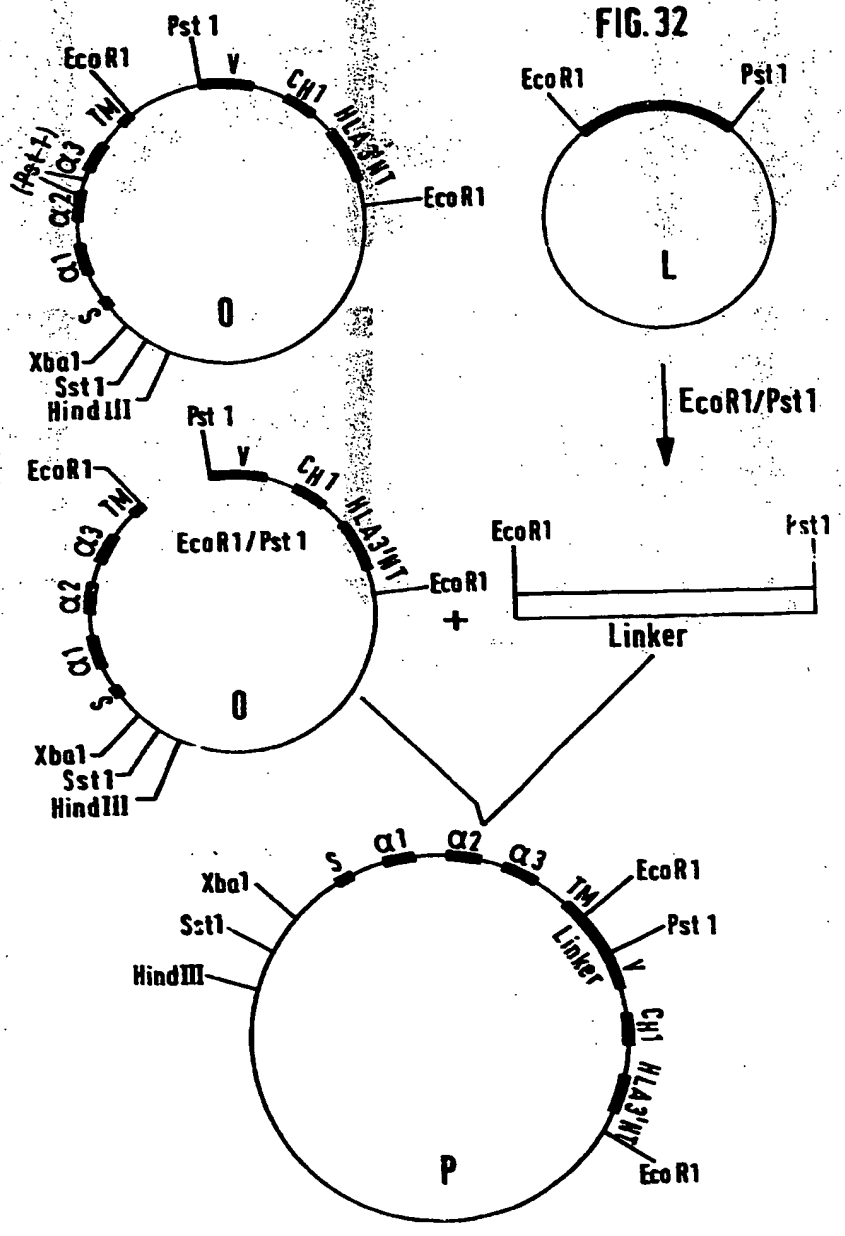

EcoR1
Pst 1
V
CH1
HLA3NT
EcoR1
TM
α3
α2
α1
S
O
Xba1
Sst1
HindIII
L
EcoR1/Pst1
Linker
+
P
Xba1
Sst1
HindIII
S
α1
α2
α3
TM
Linker
V
CH1
HLA3NT

FIG.33

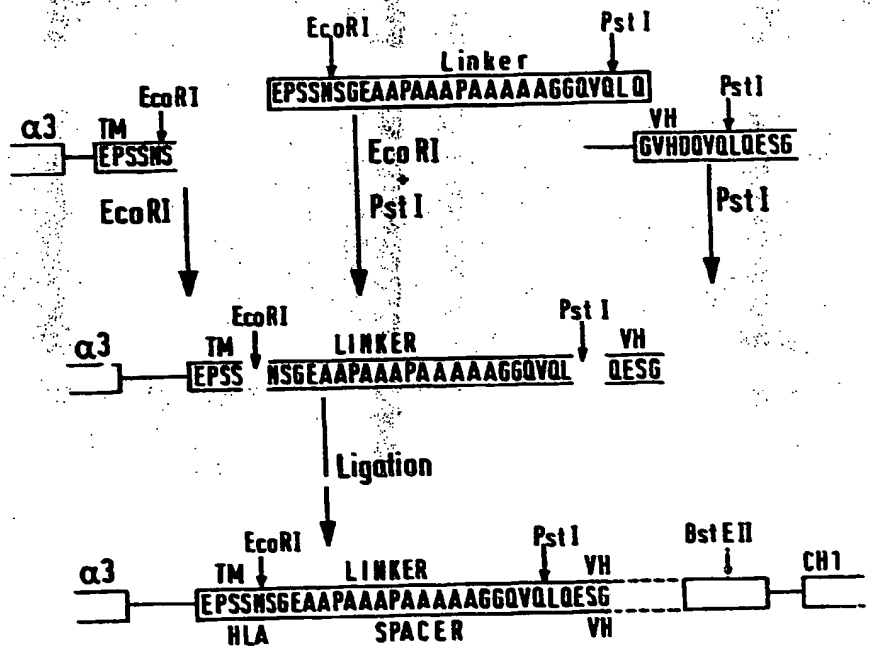
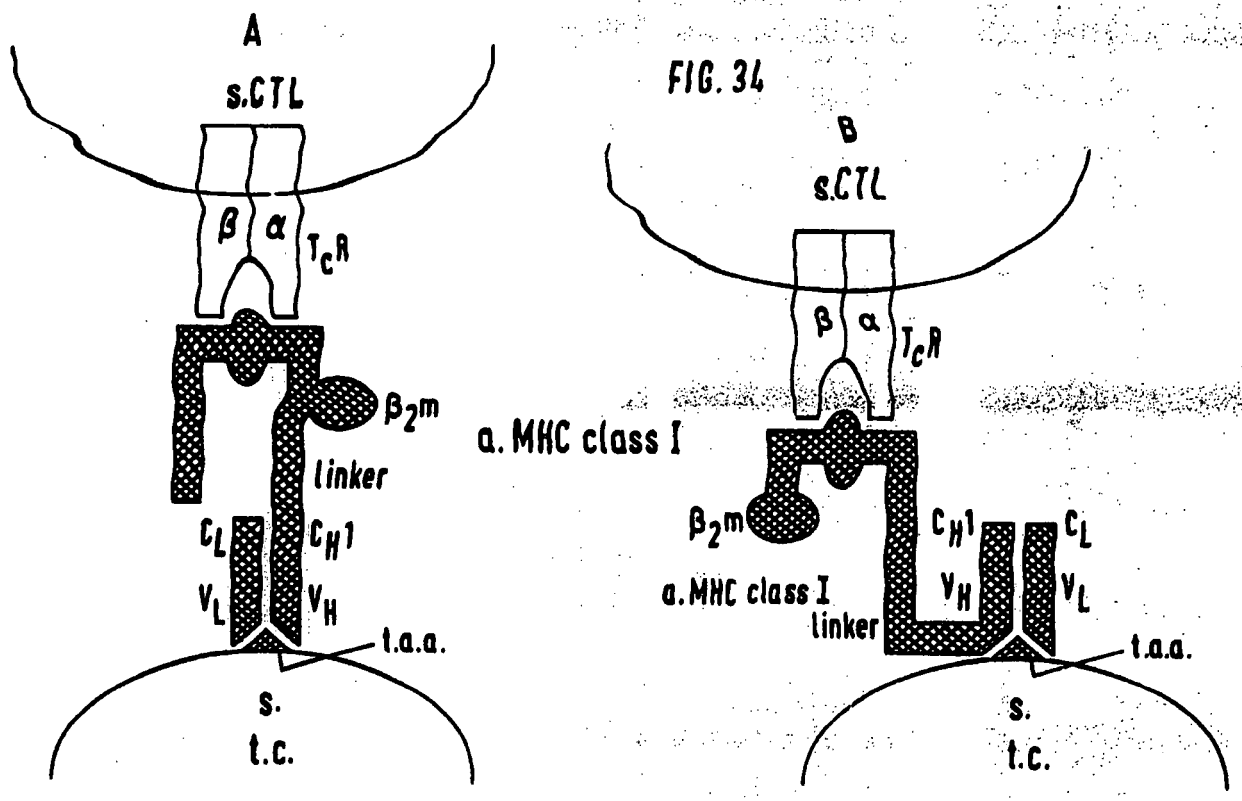

EcoRI
PstI
Linker
EPSSNSGEAAPAAAPAAAAAGGQVQLQ
EcoRI
PstI
VH
α3
TM
EPSSNS
GVHDQVQLQESG
EcoRI
EcoRI
PstI
PstI
EcoRI
PstI
α3
TM
LINKER
VH
EPSS
NSGEAAPAAAPAAAAAGGQVQL
QESG
Ligation
EcoRI
PstI
BstEII
CH1
α3
TM
LINKER
VH
EPSSNSGEAAPAAAPAAAAAGGQVQLQESG
HLA
SPACER
VH

FIG. 34


A
s.CTL
β
α
T_CR
β_2m
linker
C_L
C_H1
V_L
V_H
t.a.a.
s.
t.c.
a. MHC class I
B
s.CTL
β
α
T_CR
β_2m
a. MHC class I
linker
C_H1
C_L
V_H
V_L
t.a.a.
s.
t.c.